APETALOUS CANOLA (BRASSICA NAPUS) HYBRID SEED PRODUCTION

by

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This Thesis is dedicated to the memory of my father Bob (Robert) A. Hogarth.

A great father, bloke and farmer.

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Abstract

Canola has become an important crop in Australia as demand for its oil continues to increase and the benefits of including it in cropping rotations are recognised. During flowering canola produces an inefficient crop canopy of bright yellow flowers, which reduces radiation penetration. This results in increased leaf senescence and slower crop growth during this stage of development.

In an attempt to alleviate this effect the apetalous characteristic (absence of flower petals) was introduced into breeding lines, and incorporated into a cytoplasmic male sterility (CMS) system. The *ogura* CMS used was a newly developed system which had been introduced from radish (*Raphanus sativus*) .

This study investigated the yields obtained from apetalous male sterile canola lines, in order to determine if apetalous hybrid seed can be produced commercially. Over three years of field trials the yields obtained from apetalous male sterile lines were significantly lower than those of a male sterile petalled line also containing the *ogura* CMS. The low yields were attributed to the apetalous male sterile lines setting a low percentage of pods from potential pod sites, and very few seeds/pod.

Apetalous male sterile plants showed a strong response to hand pollination, by setting more pods and seeds/pod than plants which were not artificially pollinated, while there was no effect seen in the petalled control. This implied that the apetalous male sterile plants were not being adequately pollinated in the field by insect vectors, which in this study was primarily honey bees. Observations made in the field of bee numbers and behaviour showed that the apetalous lines attracted a similar number of bees as the petalled line, however a high percentage of apetalous flowers were 'side-worked' and therefore unlikely to be pollinated.

Pollen transfer experiments conducted in the glasshouse investigated the effect of bees side-working flowers and showed that the amount of pollen deposited on apetalous male sterile flowers was 60% lower than on flowers with petals, this appeared to be the major reason that apetalous flowers produced fewer seeds/pod.

Both the apetalous and petalled male sterile lines were found to produce fewer seeds/m^2 from each row the further it was situated from the pollen source. Logarithmic regression equations were calculated which were able to explain a large proportion of the variation in the number of seeds/m^2 between rows. It was proposed that these regression models could be useful in determining A:R line ratios for hybrid seed production blocks.

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Chapter 1.

Introduction

Canola production has increased to the extent that it is now one of the most important oilseed crops on the world market. Demand for canola oil has risen as the nutritional benefits associated with vegetable oils as opposed to animal fats have been recognised. Population expansion and economic growth in developing countries has also increased markets for high quality vegetable oils.

Until 1988 canola was known as rapeseed or oilseed rape in Australia (Colton and Sykes, 1994), and it is still known by this name in Britain. The term canola was introduced by Canadian producers to distinguish new high quality rapeseed cultivars from old cultivars not considered suitable for human consumption (Sernyk and Stefansson, 1983). Canola is defined as those cultivars of *Brassica napus* and *B. rapa* which contain less than 2% of total fatty acids as erucic acid and less than 30 micromoles of aliphatic glucosinolates per gram of oil free meal (Downey, 1990).

The canola plant produces small, round seeds which weigh from three to six milligrams. It is from this seed that oil is extracted, constituting around 40% of the weight of the seed. The meal residue remaining after oil extraction contains 36 to 40% protein (Kimber and McGregor, 1995) and is a valuable byproduct for use in animal feed rations.

Canola is the fastest growing component of the Australia oilseed industry with an estimated production area of 632,000 hectares for 1997 expected to yield on average

1300 kg/ha (Canola Association of Australia, 1997). The crop is grown in the wheat producing regions of Australia where it is included in cereal and legume rotations. It has been demonstrated that including canola in crop rotations confers a number of benefits including increased yield of subsequent wheat crops through suppression of cereal diseases, improvement of soil structure and diversification of income.

In the past canola crops have been restricted to more productive areas of the wheat belt, however the adaptation of cultivars to wider agronomic conditions and the development of herbicide resistant lines has enabled expansion into more marginal areas.

Most cultivars grown in Australia at present are 'open pollinated' which in *B. napus* means that both self pollination and some outcrossing occurs. At this stage hybrid cultivars account for around 6% of total production (G. Buzza, pers. comm.).

Current canola yields are low in comparison with some other field crops, especially cereals. This appears to be at least partly associated with an inefficient crop canopy which restricts the amount of radiation intercepted by the crop thereby limiting the production of assimilates. This research project has investigated the effect of the apetalous flower character on the production of hybrid seed, with the objective of producing a more efficient crop canopy and ultimately higher yields.

Factors Affecting Yield

Final yield in a crop is the result of a balance between available resources and the plant's genetic ability to produce seeds. Therefore it is the interaction between genetic and environmental factors which determine final yield. It is these two

elements that plant breeders and agronomists are able to manipulate in order to provide the optimal balance for the plant to be able to perform to the best of its ability in a given environment.

In order to produce this optimum, the yield determining factors must be recognised and their effect and reason for their effect must be understood. When factors such as nutrients are not limiting and the genetic effect is kept constant by the use of just one genotype it is the combination of moisture, radiation and temperature which exerts the greatest influence on yield.

Habekotte (1993) reported that slight increases in yield have been achieved for winter oilseed rape in several European countries through improved agronomy and breeding and it has become possible to obtain potential seed yields, that is seed yields defined by genetic characteristics of the crop and climatic factors. Despite this actual yields are low in comparison with winter wheat. Both crops use the same photosynthetic pathway and have similar growth periods so theoretically similar levels of production should be obtainable. Taking into account the higher energy content of oilseeds, they should be able to yield about 5.8 t/ha, about 2.5 t/ha more than is currently achieved (Habekotte, 1993).

If yields of winter wheat are used as a reference, it is clear that the efficiency of canola crops have potential for improvement, which can only be achieved by understanding the growth and development of the crop and identifying the areas which need improvement.

One of the most referred to papers in *Brassica* agronomy research by Mendham *et al.* (1981), involved a detailed investigation of the response of yield components to late sowings through the examination of patterns of growth and development. In

effect different sowing dates over several years provided a range of temperature, moisture and radiation levels. By following crop development under different conditions through to final yield, the authors were able to examine how crops responded to the various levels and combinations of yield determining factors at different stages of development. As a result they were able to identify the optimal conditions required to maximise yield and at what stage of development they were necessary.

In years which followed a typical weather pattern, yield decreased with delayed sowings, however the early sown crops were very inefficient in producing their final yield. These plants reached a large size before inflorescence initiation in winter, but were unable to support the large number of pods and seeds set due to the dense canopy they produced. The number of seeds/pod retained to final harvest was low, therefore from a high total biological yield only a small percentage was in the form of the harvestable product.

Several years over which the trials were conducted had atypical weather patterns, which affected the plants development in such a way as to make late sowings more efficient. In these atypical years yields increased with later sowings, due to the retention of more seeds/pod. These plants had a more balanced crop canopy due to delays in inflorescence initiation and flowering. The later sown crops over wintered as small plants and so inflorescence initiation was delayed until spring when the plant had produced the minimum number of leaves required. As initiation took place on smaller plants the number of potential pods was reduced which was beneficial under the conditions which followed.

Temperatures rose more quickly in the spring of the atypical years, which combined with the late initiation increased the period of growth before full flower. The size

of the plant at full flower provided an indication of its ability to realise its yield potential, because at this stage the photosynthetic capacity of the crop had reached its maximum and any further growth was dedicated to pod and seed production.

The reason that the late sown crops yielded so highly was the ability to compensate for low pod numbers by retaining a high percentage of pods and seeds/pod set at flowering. With fewer pods the canopy was more open and the effects of shading and competition for assimilates was reduced. During this period the weather conditions were favourable for plant growth, with high levels of radiation and limited water stress.

Mendham *et al.* (1981) concluded that for high yield, good vegetative growth should be followed by production of fewer pods than was produced by early sown crops. Each pod should be able to maintain near the potential number of seeds to produce high yields.

United Kingdom vs Australia

The majority of available literature concerns winter rapeseed cultivars which are sown in the northern hemisphere in autumn, become dormant over winter due to the low temperatures and are harvested in mid summer. This produces large plants with high yield potential (Mendham and Salisbury, 1995).

In Australia spring cultivars of canola are used exclusively, but crops are sown at a similar time as in Europe (late autumn), and continue growth through the mild winter and are harvested in early summer under increasing temperature and water stress. Due to the length of the growing season crops generally produce lower amounts of dry matter than winter cultivars and the major factors influencing final yield may be different to that which occurs under European conditions.

However the research conducted by Mendham *et al.* (1981) is applicable to crops grown in different environments, as it provides an indication of what factors are required to maximise yields.

Vegetative Growth

Seedling emergence comprises both germination and early seedling development. After the seed absorbs water the radicle splits the seed and the hypocotyl pushes through the soil pulling the cotyledons upwards (Colton and Sykes, 1994). Temperature, light and water are the major environmental factors determining the success of germination and early seedling development (Nykiforuk and Johnson-Flanagan, 1994). As is the case throughout the plant's life-cycle, temperature levels determine the rate of development and good establishment is crucial as it enables the crop to achieve maximum leaf cover in the least time allowing optimal radiation interception. Successful germination also depends on genotype and storage conditions experienced by the seed, and is also affected by the environmental conditions in which its parent plant grew and matured (Gutterman, 1980 cited in Acharya *et al.*, 1983).

After expansion of the cotyledons, which turn green after exposure to light, the leaves form a rosette. The stem apex continues to produce leaf primordia until inflorescence initiation, but leaf initiation rates are faster than leaf appearance so leaf primordia accumulate around the apex (Mendham and Salisbury, 1995). Unless there is competition for light, there is little or no stem extension until after inflorescence initiation (Buzza, 1979).

Flowering begins on the mainstem with flowers from the base of the raceme opening first followed by flowers positioned towards the apex. Buds in the axils of the leaves on the mainstem develop into primary branches progressively towards the base of the plant, with flowering beginning on the first primary branch and continuing on to the later formed branches. Primary branch number is therefore determined by the number of leaves produced before inflorescence initiation as they form branches in the leaf axils. Secondary branches can also develop in the axils of the bracts on the primary branches, but in most crops do not produce many pods with seeds (Mendham and Salisbury, 1995).

A well grown crop produces a thick canopy of yellow flowers. Each flower consists of four sepals, four petals, an outer whorl of two short stamens, an inner whorl of four long stamens and a superior ovary of two united carpels, surmounted by a style with a lobed stigma. There are four nectaries, two inside the bases of the short stamens and two outside the ring of long stamens (Free and Nuttall, 1968).

Pollination

Canola is generally a self-pollinating plant, though in natural conditions up to 40% outcrossing can occur, depending on environmental conditions and to some degree genotype (Becker *et al.,* 1992). Outcrossing occurs through insect vectors which deposit pollen onto the stigma while gathering nectar or pollen, and may also occur via wind and physical contact between flowers. Self pollination usually occurs between anthesis and one day after anthesis as the style extends through the ring of anthers (Morrison, 1993).

The crop is highly attractive to honey bees which are the main pollination vectors in most instances. While bees may not be necessary in canola grown for oil, for hybrid seed production they are essential and hives are placed in all crops grown for hybrid seed in Canada and Tasmania.

Factors Affecting Flowering

The vernalisation and photoperiod requirements of canola are important in matching a cultivar with climate in order to optimise yield. Under European conditions yield can be increased by prolonging the vegetative stage and thus increasing the size of the inflorescence, while still allowing adequate time for flowering and seed ripening before the end of the growing season (Friend, 1985). Such conditions may also produce an inefficient plant canopy so a balance is required. In other environments it may be more important to use early flowering and maturing cultivars which enable the crop to avoid water and temperature stress. The large variability of genetic material available in *Brassicae*, has provided a wide range of vernalisation and photoperiod responses. This has enabled the crop to be grown in a variety of environments, from hot, water stressed conditions experienced in India and Australia to colder climates such as Europe and Canada.

This discussion will mainly involve spring cultivars that are used exclusively in Australia.

Temperature, photoperiod, genotype and possibly a vernalisation response influence the rate of development of spring cultivars. While some spring cultivars still have a vernalisation response it is not essential for flowering as in winter cultivars. These factors result in different times to flowering in cultivars depending on sowing time

and in what region the crop is grown (Salisbury and Green, 1991). An understanding of what influence these factors will have on time to flowering and time to harvest is important in order to provide the optimal conditions for a given stage of development in a particular environment. If one of the factors that influence flowering is not fulfilled and it is delayed, seed development may take place under increasing temperature and water stress.

The change from vegetative to reproductive growth occurs initially in the apex of the mainstem where a floral primordium is produced in the axil of the last leaf primordium (Mendham and Salisbury, 1995). The change occurs once a minimum number of leaves have been produced and vernalisation and photoperiod requirements have been fulfilled.

In winter cultivars it is possible for the plant to reach a large size before flower initiation occurs if it is delayed by vernalisation or photoperiod responses. Larger plants have more leaf axils in which to produce branches and flower primordia, however the realisation of this yield potential is highly dependant on conditions post-flowering, as previously discussed.

In spring cultivars inflorescence initiation occurs once the plant produces the minimum number of leaves, and any photoperiod or small vernalisation requirements are satisfied. This is further modified by temperature, with higher temperatures causing flowering to occur sooner (Salisbury and Green, 1991), but in a similar thermal time or number of day degrees. Thus spring cultivars undergo continuous development without the delays associated with winter cultivars. The overall effect is to produce plants in which inflorescence initiation occurs sooner in smaller plants resulting in a reduced yield potential.

Seed and Pod Development

There is a clear progression in the development of plant organs, each phase nearing completion before the next begins. In some situations which cause extended flowering and branching there may be a greater degree of overlap. Mendham *et al.* (1981) described how when stems and branches finish growth, pod hulls begin rapid expansion and significant increases in the dry weight of seed is delayed until the pods have approached their maximum weight.

During initial stages of this important phase of development when demand for assimilates is at its greatest, plants are still flowering heavily which results in the senescence of leaves and the abortion of pods and seeds. Later as the pod canopy develops, flowering finishes and more radiation is available to the photosynthetically active pod surfaces, however the pod canopy itself causes shading of lower pods and results in a reduction in the number of seeds they carry through to harvest (Rao *et al.*, 1991). The effects of the flower and pod canopy are more pronounced in early sown winter crops which reach a large size by flowering and produce large numbers of pods as previously described by Mendham *et al.* (1981).

Assimilate Distribution and Sink Strength

Major and Charnetski (1976) looked at the origin and destination of ^{14}C-labelled assimilates in oilseed rape plants. The results indicated that leaves, stems, and pods were capable of assimilating $^{14}CO_2$ but only leaves and stems exported assimilate to other organs. The components to which assimilate were exported included roots, pods, seeds, apices and infertile pods. At the stage of development when the

experiment was conducted, (lower pods starting to fill), it appeared that apices and seeds were the strongest sinks. It was observed that infertile pods were still incurring a cost to the plant by drawing on assimilates even though they contained no seeds.

Assimilate distribution was examined in more detail by Keiller and Morgan (1988), who conducted experiments to analyse activity within individual organs at different stages of development. They found that while buds and newly opened flowers were strong sinks, mature open flowers and young pods were weak sinks. It appeared that flowers did not regain their strong sink strength until well after fertilisation when the level of cellular activity increased.

The results indicated that during the critical stage of seed set the sink status of flowers is low until the ovules are fertilised and seed development begins. As previously mentioned, at this stage during flowering the dense mass of flowers shade any remaining leaves so the actual amount of assimilate produced would be expected to be quite low. The leaves are virtually non-functional and any treatment which enhances assimilate supply during the flowering period should improve seed set.

Keiller and Morgan (1988) also found that the sink strength of the apices falls from high to low around 10-16 days after anthesis and occurs at approximately the same time on all branches. It was suggested that the cessation of apical growth was the result of a change in the overall carbon budget balance within the plants from being in excess to deficient, with the strong sink strength of the pods enabling them to monopolise the diminishing supply.

Other workers have suggested the mechanism is more hormonal, however Farrington and Pate (1981) and Binne and Clifford (1981) both cited in Keiller and

Morgan (1988), concluded that it was not possible to differentiate between nutritional and hormonal factors in the inhibition of pod and flower formation. Whatever the mechanism it is apparent that once rapid embryo development and oil deposition starts in the oldest pods, apical development ceases.

Keiller and Morgan (1988) also demonstrated to some extent why lower order branches are less productive than higher order branches. As apical development finishes at around the same time in all branches the lower order branches have a shorter time in which to achieve pod and seed formation. The end result is that progressively more assimilates are invested in stem and pod growth on the lower branches as opposed to seed and pod development. In effect the plant is wasting assimilate in producing unproductive structures which could be better used in increasing the number and size of seeds in pods of higher order branches.

Due to the sequential development of reproductive organs during flowering there is intense competition for assimilates and the plant must achieve a balance for partitioning resources between developing seeds and pods and flowering on lower branches. Due to absorption and reflection of sunlight by flowers at this stage, and the development of a dense pod canopy, leaves senesce and assimilate production mainly occurs in young stems and pods. While these organs are capable of photosynthesis, they are not as active as leaves and possess fewer stomata (Major, 1975).

Increasing Crop Efficiency

Mendham *et al.* (1981) demonstrated how competition for resources resulted in only 50% of potential pods surviving to produce seeds in early sown crops which had a

dense pod canopy. Even in later sown crops with a more open canopy only slightly more than half survived.

There have been reports of the benefits associated with a more open canopy structure as a result of low plant density crops. Jenkins and Leitch (1986) demonstrated how lower biomass, fewer pods/m^2 and lighter seeds of late sowings were compensated for by the retention of twice as many seeds/pod. McWilliam *et al.* (1995) reported that although crops with high plant numbers intercepted more radiation between flowering and harvest than sparse crops, it was used less efficiently than prior to flowering. Mendham *et al.* (1990) also reported a similar result, with an oilseed rape crop producing 1.5 g of dry matter per MJ of intercepted radiation pre-flowering and less than 1.0 g of dry matter per MJ after flowering. Higher pre-flowering efficiency is explained by the presence of young, active leaves which possess more stomata per unit area than pods and stems (Mendham, 1995), which are the main photosynthetic organs after flowering. Leaves are lost due to shading and reflectance of light by flowers and developing pods. In a crop with a low plant density more light is able to penetrate to the lower layers of the canopy enabling leaves to persist for a longer period. Due to this factor as well as less competition from fewer pods, a higher number of seeds/pod are retained and sparse crops are able to yield better than would be expected. However low density crops are potentially a high risk method of increasing radiation penetration if the conditions for plant establishment are not optimal, or if weed control is not adequate.

Recent attempts to increase the efficiency of the crop canopy without reducing plant numbers have included the use of erectophile pods and the apetalous flowering characteristic.

It was proposed by Rao and Mendham (1991) that the upright pod character of 'chinoli' (*Brassica campestris* subsp. *oleifera* x subsp. *chinensis*) would result in better pod filling due to improved distribution of light in the pod canopy. This has been demonstrated to occur with the erect leaves in cereal canopies (Yoshida, 1972 cited in Rao and Mendham, 1991). However, poor agronomic characteristics of the chinoli line did not give the upright pod character an opportunity to demonstrate any of the proposed benefits.

The potential advantage of increased light transmission with erectophile pods was investigated through the comparison of a *B. napus* line with this character and two commercial cultivars by Fray *et al.* (1996). The erectophile pod variety used, N-5-130, produced pods angled 20-25° further from the horizontal than the commercial cultivars. N-5-130 reflected less incident Photosynthetically Active Radiation (PAR), than the two commercial cultivars and increased the amount of radiation reaching the base of the pod canopy. This allowed N-5-130 to retain more seeds/pod in the lower levels of the canopy, while the conventional cultivars had fewer seeds/pod and were unable to compensate by producing heavier seeds as less assimilate was produced by the lower pods.

It was suggested that the incorporation of the erectophile pod character into cultivars with a compact canopy architecture may improve seed yield production by increasing PAR availability to pods in the lower horizons. However the erectophile pod character would appear to be more beneficial when introduced into genotypes producing a deep pod canopy, when increased PAR transmission could maintain higher seed yields at the base of the canopy, thereby increasing overall crop yield (Fray *et al.*, 1996).

Mendham *et al.* (1981) noted that during flowering a large number of flowers and pods were aborted. This occurred during a critical stage in the growth and development of these components when yield potential was set. During this period of flowering it was also observed that the bright yellow petal canopy absorbed or reflected up to 60% of incoming solar radiation, thus depriving leaves lower in the canopy of light. This resulted in the amount of assimilate available to developing pods and seeds being reduced and was believed to be the cause of the high rate of pod and seed abortion.

With this in mind Buzza (1983) developed an apetalous canola line, after observing plants with reduced petal numbers during plant breeding trials. The flowers of these plants were normal except for the absence of petals (figure 1.1), though occasionally flowers with one or two petals were produced. The anthers were found to be slightly shorter than many cultivars, but still well in the range found in canola cultivars.

Figure 1.1. Apetalous flower (right) showing normal flower appearance apart from the lack of petals, in comparison with a conventional cultivar (left).

Rao *et al.* (1991) using an apetalous line obtained from Buzza (1983), compared it with a conventional Australian spring cultivar 'Marnoo' to investigate the physiological significance of the apetalous character on radiation distribution, leaf persistence, yield and components. The apetalous line was morphologically similar to 'Marnoo', but the flowers were larger and produced longer and wider pods. No problems were observed in the pollination of the apetalous flowers with similar numbers of bees observed on both lines.

The apetalous line transmitted a greater percentage of incoming radiation to all heights within the crop canopy than 'Marnoo' with the greatest difference (30%) occurring at the base of the inflorescence. This enabled the apetalous line to produce a higher Leaf Area Index (LAI) which was also maintained for a longer period of time.

The apetalous line yielded slightly more than 'Marnoo', but on fewer plants due to poor germination and with fewer pods/m^2. The most significant difference in yield components was the greater number of seeds/pod in the apetalous line compared with 'Marnoo'. The apetalous flower character appeared unstable in higher temperatures and long days with later opening flowers, particularly of late sowings producing one to two residual petals. This was not considered to be of physiological or agronomic significance as the apetalous characteristic would mainly be of benefit during peak flowering, but it would make the maintenance of pure seed lines more difficult (Rao *et al.*, 1991).

Röbbelen (University of Göttingen, Germany) also developed apetalous lines, and these were used by Fray *et al.* (1996) in research on canopy modification.

While these apetalous lines increased radiation penetration during peak flowering, the character was not stable and the apetalous line was of generally poor agronomic standard, so they did not yield as well as the erectophile pod line discussed previously, or two commercial cultivars. The low yield was the result of a low number of productive pods/m^2 and seeds/pod. Data for top dry matter production was not presented so it is unclear if the low number of productive pods was a result of poor vegetative growth. However the apetalous line did not produce as many potential pods as other lines in the study.

The results from both Fray *et al.* (1996) and Rao *et al.* (1991) indicated that improving the light distribution through the crop canopy is possible, and beneficial in plants with a good agronomic background. The apetalous character reduced the effect of light reflection and absorption during flowering, and the erectophile pod character assisted in light penetration through the pod canopy when pods were the main source of assimilates.

The introduction of these characters either in combination or separately into agronomically superior lines should enable higher yields to be achieved, hopefully approaching the theoretically obtainable levels proposed by Habekotte (1993) of 5.8 t/ha for winter cultivars.

Hybrid Seed Production

The advantages gained from cross pollination on the performance of subsequent offspring have been recognised for many years. Charles Darwin in his book *"Cross and Self-Fertilisation of Plants"*, published in 1891, acknowledged the abundant evidence that the flowers of most kinds of plants are constructed so that

occasionally or habitually cross-fertilisation takes place. He described some of the mechanisms by which plants achieve this, such as self incompatibility, dioecious and monoecious plants, and heterostyly. He realised that the diverse and effective means that plants had evolved to ensure cross-pollination was significant evidence that they derived some advantage from the process.

"We should always keep in mind the obvious fact that the pollination of seed is the chief end of the act of fertilisation; and that this end can be gained by hermaphrodite plants with incomparably greater certainty by self-fertilisation, than by the union of the sexual elements belonging to two distinct flowers or plants. Yet it is as unmistakably plain that innumerable flowers are adapted for cross-fertilisation, as that the teeth and talons of a carnivorous animal are adapted for catching prey; or that the plumes, wings, and hooks of a seed are adapted for its dissemination." (Darwin, 1891).

Darwin attempted to determine the reason for this adaptation by examining whether the seedlings from cross-fertilised flowers were in any way superior to those from self-fertilised flowers. Over 11 years he carried out experiments on over 13 different genera, in which he compared plants produced from self-fertilised seed and seeds produced by hand crossing flowers with pollen from another plant of the same variety. One of the plants used was the common cabbage, *Brassica oleracea,* which he noted were adapted for cross-pollination, or self-pollination should this fail.

"It is well known that the varieties [of B. oleracea] *are crossed so largely by insects, that it is impossible to raise pure kinds in the same garden, if more than one kind is in flower at the same time."* (Darwin, 1891).

Initially Darwin's comparison between selfed and crossed seedlings was based on height, however he found the difference between the two treatments to be quite small with the crossed plants being on average only 2.01 inches taller. He recognised that this measurement did not reveal the vast superiority of the crossed over the self-fertilised plants. Therefore the plants were harvested and weighed, the eight crossed plants weighed 219 ounces, while the self-fertilised plants weighed only 82 ounces.

Darwin continued the experiments with subsequent generations, continually inbreeding the selfing line while introducing "fresh stock" to the crossed plants and found that in the next generation the difference was even greater. *"This difference shows in the clearest manner the enormous benefit which the plants derived from a cross with another plant belonging to the same sub-variety"* (Darwin, 1891).

While Darwin recognised the increased vigour of hybrid plants he was unable to explain the reason for the greater productivity. With the development of genetics increased vigour of the F_1 generation has become known as heterosis. The increase in vegetative growth and yield obtained from hybrids is due to heterosis from cytoplasmic and chromosomal diversity as a result of more heterozygous loci, and a lack of inbreeding depression which enhances the ability to adapt to a challenging environment (Lefort-Buson and Dattee, 1982).

Interest in *B. napus* hybrids was stimulated by reports of heterosis for seed yield in F_1 generation plants of 40-60% over their parents, from hybrid seed produced by hand pollination and anther emasculation (Sernyk and Stefansson, 1983). Lefort-Buson and Dattee (1982) measured hybrid vigour for yield to evaluate the potential of oilseed rape hybrid cultivars. They found that heterosis affected the whole plant with hybrids being superior in vegetative growth and yield components such as

pods/plant, seeds/pod and ultimately seed yield, though the differences varied greatly according to parents and the season. Other workers have reported that yield increase in F_1 hybrids was the result of more productive pods produced, with no significant differences in other yield components (McGee and Brown, 1995; Falk *et al.*, 1995). Lefort-Buson and Dattee (1982) concluded that economic interest in rape hybrids was justified, as some parent combinations yielded up to 50% more than the best parent.

It is important that the amount of heterosis exhibited is enough to justify the increased cost of hybrid seed production, because simply crossing two lines will not necessarily result in a hybrid effect resulting in higher yields. Hybrid parents are required which are genetically different and are able to combine well to produce high yielding hybrids with good agronomic traits (Busch, 1995). It is generally recognised that the most productive hybrids are produced from crosses between genetically diverse cultivars (Brandle and McVetty, 1990; Bartkowiak-Broda *et al.*, 1995; Falk *et al.*, 1995).

Brandle and McVetty (1990) proposed that in the case of spring oilseed rape there appears to be three genotypic groups, which are related to the genetic origin of their parents. These origins are Asian, European and Canadian and it appeared the Asian heterosis group was more distinct from the Canadian and European groups which overlapped to some extent, resulting in only some crosses between the two showing a heterotic effect. In contrast all the crosses made between an Australian cultivar 'Marnoo', (with Asian parentage) and European and Canadian groups showed heterosis for yield.

A hybrid seed production system must prevent self-pollination and ensure cross-pollination between the parent lines. This has been possible in crops such as maize

by physically removing the male flowers that could be done mechanically or by hand due to the production of separate male and female inflorescences. While hand emasculation may be viable in high value crops with perfect flowers such as tomatoes, it would be uneconomic in high volume, low value crops such as canola, so biological methods are required to prevent selfing and to control cross pollination by insects or wind.

Such mechanisms exist naturally in some plant species and include self incompatibility (SI), nuclear male sterility and cytoplasmic male sterility (CMS). Male sterility occurs as abnormalities which may not survive in nature, but SI is a common system in natural populations.

The pollination control system used in this study was CMS and is one of the most favoured systems for hybrid seed production. The three lines required for the system are relatively easily maintained and multiplied for production. In the SI system, by contrast, difficulties can be experienced in producing self fertilised seed. Treatment of SI lines with salt water solutions or high CO_2 atmospheres may be necessary to break the SI system (Grant *et al.*, 1991). Any seed which is produced may be of limited quantity and poor quality making the system expensive and unpredictable on a large scale. Small scale production of SI based hybrids is carried out for diploid, SI *B. oleracea* vegetable and fodder crops.

Cytoplasmic Male Sterility (CMS) in B. napus

The genes that control CMS are contained within the cytoplasm and result in male sterile plants which should not produce pollen but are able to set seed if another pollen source is available. It appears that CMS results because of a multitude of

effects, all more or less related to insufficient or mistimed supply of necessary resources for developing microspores (McVetty, 1997). A CMS system is comprised of three lines, the male sterile A line just described, the B line (maintainer) and restorer or R line. The A and B lines are identical apart from the A line containing a gene for sterility in the cytoplasm which prevents it from producing pollen, while the B line with a gene for fertile cytoplasm is able to self pollinate. The R line is able restore fertility to the A line as it contains nuclear genes carried by pollen which 'over-ride' the male sterility caused by the cytoplasm. In the case of *Ogura* CMS there are two dominant genes which restore fertility. The restorer genes temporarily suppress the expression of CMS by mechanisms not well understood, permitting normal or near-normal pollen production (McVetty, 1997). The A and R lines are the parents which are combined to produce an F_1 generation possessing heterosis for yield, so these lines must have good combining ability. The B line is necessary to produce more A line seed, the seed produced from an A line with the B line as a pollen parent will be male sterile as the cytoplasm characteristics can only be inherited maternally. For a more detailed description of CMS see Buzza (1995).

At this stage only the *Polima* CMS (*pol* CMS) system has been used to produce commercial hybrids (Buzza, 1995). This CMS system was found in spring rape in China by Fu (1981) cited in Bartkowiak-Broda (1995), and was used to produce all registered hybrids in Canada in 1995 (Bett and Seguin-Swartz, 1995). However there are some problems with the system, which make it difficult to produce 100% hybrid seed under all conditions. One of the major problems with *pol* CMS is the instability of male sterility under high temperatures or high levels of nitrogen fertilizer (Bartkowiak-Broda, 1995). With breakdown of male sterility, blisters of pollen are produced on A line anthers which allow self pollination. While the

pollen produced by the A lines was shown to have a lower viability than pollen produced on B lines (Gurjeet and Banga, 1995), self pollination is still a possibility resulting in contaminated hybrid seed.

A significant breakdown of the *pol* CMS system occurred in Tasmania, Australia in the summer of 1994/95, causing a severe disruption in the production of Pacific Seed's hybrid seed. Several unseasonable days of temperatures greater than 30°C during flowering resulted in blisters of pollen being produced on the A line plants. Once this was realised the crops which were flowering were cut back to avoid seed contamination and allowed to reflower under cooler conditions which resulted in significantly lower yields (Mendham, pers. comm.). Trials containing *Ogura* CMS plants which were flowering at the same time showed no evidence of pollen production.

Some progress has been made in regards to this problem, with Gurjeet and Banga (1995) reporting *pol* CMS male sterile lines which were stable at high temperatures. Although it has been possible to select hybrids with *pol* CMS cytoplasm which show heterosis for yield, it has been shown there is a high biological cost associated with the *pol* CMS cytoplasm which affects not only yield, but in some cases oil and protein levels as well (McVetty *et al.*, 1990).

Ogura cytoplasmic male sterility (*ogu* CMS), discovered in radish (*Raphanus sativus*) by Ogura cited in Thompson (1983), is one of the most promising CMS systems for the production of hybrid seed in canola. The main benefits include its stability over a wide range of environmental conditions and the development of good female and maintainer lines. The system is still not in commercial production however, as the restorer genes were found to be closely linked to high glucosinolate

levels. Plant breeders have only recently been able to break this linkage (Buzza, 1995).

The *ogu* CMS was introduced into *B. napus* by transferring the nucleus of *B. oleracea* into radish cytoplasm by Bannerot *et al.* (1975) cited in Thompson (1983), by crossing the two species, followed by backcrossing to *B. napus.* The resulting *B. napus* plants were backcrossed by Rousselle (1979) cited in Thompson (1983), with many different oilseed rape cultivars which produced lines with good male sterility under a wide range of environmental conditions. However the leaves of the CMS plants were chlorotic under low temperatures, which was attributed to radish DNA located in the chloroplasts. To eliminate this Pelletier *et al.* (1983) fused protoplasts from different lines which produced a number of different cybrids, that is hybrid cytoplasm with components from both donors.

These cybrids produced plants with a range of characteristics, some of which did not show the chlorophyll deficiencies or low nectar production of previous lines (Pelletier *et al.* 1983). Cybrid 58 produced by this protoplast fusion was used by Pacific Seeds to develop the CMS lines used in the present study. It contains the chloroplasts of *B. napus* and *B. napus/R. sativus* recombined mitochondria, which contains the CMS genes.

Other cybrids produced possessed temperature modified characters, and some were not completely male sterile. In a cybrid studied by Polowick and Sawhney (1987) the anthers showed instances of feminisation under low temperatures, where external ovules capable of being fertilised were produced, as well as stigmatic surfaces with identical papillate cells as those found on a normal stigma.

Cybrid 58 did not exhibit any of these characters and produced male sterile flowers possessing stamens which while shorter than wild type flowers appeared normal with completely desiccated and shrunken anthers containing no pollen under any treatments. Gourret *et al.* (1992) proposed that the reason for the characteristics of cybrid 58 was due to its mitochondrial DNA being closer to that of rapeseed, while the other cybrids contained higher levels of radish DNA. Thus the protoplast fusion procedure conducted by Pelletier *et al.* (1983) eliminated the undesirable characteristics formerly associated with the *ogu* CMS, while maintaining the high degree of male sterility.

Gourret *et al.* (1992) also explained why no pollen was produced in the CMS line. It was the result of excessive vacuolization of tapetal cells leading to their degeneration prior to the sudden collapse of the microspores. These observations were in agreement with the data obtained by Bartkowiak-Broda *et al.* (1979) cited in Gourret *et al.* (1992) and corroborate many features noted by Polowick and Sawhney (1991a,b) cited in Gourret *et al.* (1992). As well as the lack of pollen production, dehiscence of the stamen fails to occur.

A suggested reason for the mechanism of male sterility was proposed by Singh (1995), who discovered that the stamens of an *ogu* CMS line had reduced levels of active cytokinins and higher levels of abscisic acid in comparison with a conventional cultivar. It is possible that the altered levels of these hormones may be responsible for the prevention of pollen production.

In male sterile plants, expression is generally restricted to stamen and pollen development, however Singh and Sawhney (1992) reported that *ogu* CMS plants had lower rates of germination and lower seed size and weights in comparison with a normal line. These differences were attributed to changes in endogenous

hormones which may have been caused by the presence of radish mitochondrial material present in the plants containing the *ogu* CMS cytoplasm. Lower levels of cytokinins were also detected in leaves and roots of the *ogu* CMS line. The effect that this may have on crop establishment and subsequent development was not discussed.

As no restorer genes for the *ogu* CMS were identified in *B. napus* lines, they had to be introduced from the original source. This was accomplished by creating a *Raphanobrassica* amphidiploid (2n=56), by crossing *R. sativus* carrying the restorer genes with *B. napus*. Intergeneric crosses were then made between the *Raphanobrassica* and male sterile rapeseed with the *ogu* type cytoplasm (Heyn, 1976 cited in Delourme and Eber, 1992). Restored *B. napus* plants were selected from the progeny of this cross (Pellan-Delourme *et al.*, 1987). The most promising restored plants were obtained from cybrids 27 and 58 (Delourme *et al.*, 1991), cybrid 58 being the basis of Pacific Seeds material as previously discussed.

There were however some problems with the restored material in that it set a low number of seeds, which on further investigation was found to be due to a high rate of embryo sac abortion. Delourme and Eber (1992) proposed that the reason for the low female fertility was the retention of additional radish genetic information by the restored rapeseed plants in addition to the restorer gene. As a result a breeding program was undertaken to try and eliminate the unfavourable radish information and increase female fertility.

Improvement in the female fertility of the restored material was achieved through continued self pollination, backcrosses with canola quality rapeseed lines and test crosses on the most promising male sterile cybrids. In 1989 an improved family

was selected, giving rise to progeny with good female fertility (Delourme *et al.*, 1995).

Delourme *et al.* (1995) showed the female fertility of the restored material to be significantly higher than the unimproved controls and not significantly different to standard cultivars, however the actual number of functional ovules present was not given. Although the problem of female fertility appeared to be solved, there were difficulties encountered in producing R lines with glucosinolate levels lower than 25 micromoles. However the detection of canola quality restored plants reported by Delourme *et al.* (1995) and Buzza (1995) in spring and winter material indicates that this linkage may have been broken.

Aim of Research

Preliminary trials of apetalous *ogu* CMS A lines using the respective B lines as the pollen source, produced yields up to 1300 kg/ha (based on total area), which was considered to be economically viable (Hogarth, 1993). The three apetalous A lines used in this trial had good agronomic characteristics and showed no evidence of pollen production. Despite these trials being sown late in the season they experienced favourable weather conditions for plant growth and pollination during flowering. There were some indications that the male sterile apetalous flowers had some difficulty in successfully opening, however the effect which this had on final yield was not determined.

The aim of the present research project was to study in more detail the yield and yield components of several apetalous A lines. In order for the apetalous lines to be released commercially it was necessary to determine how they performed under different environmental conditions. It was also intended to identify any limiting factors for yield in the apetalous lines, and the reason for any such problems so they could be alleviated in any future development.

The first set of trials presented in chapter 2 examines the yield potential of apetalous male fertile (B) lines in both spring and autumn sowings. Also included in this chapter is a trial which evaluated the yield of hybrid seed produced by a range of apetalous male sterile (A) lines, from which lines were selected for further investigation.

Chapter 3 concentrates on determining what yield components were chiefly responsible for the lower yields produced by the apetalous A lines, in comparison

with a conventionally petalled line. The following chapter attempts to explain why the apetalous lines suffered from poor pod and seed set through detailed examination of bee behaviour and hand pollination experiments.

Chapter 5 looks at the effect which temperature had on flower opening and development, and offers a possible explanation for the high number of aborted pods produced by the apetalous A lines. This chapter also looks at the pollen loads of A line stigmas after exposure to honey bees and a pollen source.

The data produced from two seasons of trials is examined in chapter 6, relating yield and yield components to the position of a plant in relation to the pollen source. Using this data regression models were developed which were able to explain a large percentage of yield and seed number variation.

The performance of both petalled and apetalous A lines was examined over three seasons of trials through a combined analysis. This allowed comparisons to be made of the lines under different environmental conditions.

The data presented in this thesis will be used to test the hypothesis:

"Economically viable yields of hybrid seed are obtainable from Ogura *cytoplasmic male sterile lines which are also apetalous"*

Chapter 2.

Two trials were conducted in the 1994/95 season, consisting of a B line trial (trial 1) with autumn and spring sowing times, and a spring sown hybrid seed production trial (trial 2). Trial 1 was used to establish the agronomic potential of the male fertile apetalous lines, while trial 2 investigated the effect of combining the cytoplasmic male sterility and apetalous characters on hybrid seed yield.

Trial I

Comparison of Apetalous and Petalled B lines

Objectives

The objective of this trial was to compare yields of self-fertile, apetalous lines with those of conventional cultivars. Ideally F_1 seed from an A line-R line cross would have been used in this trial, but at this stage *Ogura* canola quality R lines were not available, therefore B line material was used. Normally the B (maintainer) is used to produce male sterile A line seed from an A line x B line cross. Previous canola trials conducted in Tasmania indicated that higher yields were obtainable from autumn sown crops in comparison with those sown in spring (Mendham *et al.*, 1984; Mendham *et al.*, 1990). Therefore trials were sown in autumn and spring. Spring is the usual sowing time for hybrid seed production in Tasmania, as this results in flowering during favourable weather conditions for bee activity and hence pollination. Spring sowing also complements northern Australian production that occurs over the Tasmanian winter. Several planting densities were included in the

trial as Rao (1988) had reported the ability of apetalous plants to retain more seeds/pod at higher densities than did the conventional Australian spring cultivar 'Marnoo'.

Introduction

Trials conducted in Tasmania by Mendham *et al.* (1984) using Australian spring canola cultivars produced seed yields up to 5.5 t/ha from an irrigated autumn sowing and 4 t/ha from an irrigated spring sowing. At that time the yields of the autumn sowing were the highest reported anywhere for canola. This indicated the potential for high canola yields in Tasmania where mild winters permitted continual growth, and moderate temperatures during spring and early summer provided a long period for seed development.

One of the highest yielding cultivars from these trials, 'Marnoo', produced a very efficient crop canopy by setting a moderate number of pods (8240/m^2), while still producing a large amount of top dry matter. This enabled plants to retain up to 20 seeds/pod and produce high yields. The ability to retain seeds by a given cultivar is an important character for high yields, as the variation in the number of seeds/m^2 normally explains most of the yield variation across cultivars for a range of conditions (Mendham *et al.*, 1984). The characteristics of the high yielding cultivars were similar to some late sown crops described previously by Mendham *et al.* (1981), which produced higher yields than early sown crops in atypical seasons. A similar relationship to that established by Mendham *et al.* (1981) between the dry weight of the crop at full flower and the number of seeds/pod retained was

demonstrated in this study. Nevertheless some differences in yield components was shown to be due to the inherent seed retention ability of a given cultivar.

These trials indicated that the factors controlling yields of late sown crops in the U.K were also operating on Australian cultivars in the milder Tasmanian climate. This was despite the fact that the Australian cultivars were spring types and were able to grow more rapidly at low temperatures compared with European lines, a characteristic attributed to Japanese parentage of the Australian lines.

In a set of experiments comparing a series of irrigated crops sown from autumn through to spring Mendham *et al.* (1990) looked at the effect of delayed sowing on Australian spring type cultivars. Yields were found to generally decrease with later sowings, which was the result of a reduction in the number of pods produced, partly offset by an increase in the number of seeds/pod. In contrast to the findings of Mendham *et al.* (1981) for U.K cultivars and conditions, growth before flowering in these trials was not a limiting factor as late sowings reached similar levels of dry matter production as early sowings. However the subsequent growth of late sowings after flowering was limited, while early sowings made up to 60% of their growth after this period. Therefore the benefit of early sowings was a longer period for seed development, while delaying sowing resulted in the crop undergoing more rapid development under higher temperatures and longer days, restricting the number of pods produced.

Rao *et al.* (1991) investigated the effect of the apetalous characteristic on radiation distribution, leaf persistence, yield and components in comparison with the petalled cultivar 'Marnoo' under Tasmanian conditions. The trial included a plant density treatment. While the early growth of the two lines was similar, the apetalous line was able to maintain leaf cover for a longer period at both densities. Even at the

high plant density treatment of 133 plants/m^2 the apetalous line was able to maintain at least some leaves throughout the flowering period.

Increased radiation penetration into the plant canopy enabled the apetalous line to produce secondary branches at all densities, while 'Marnoo' had no secondary branches at high plant density. The production of more branches and the retention of more seeds/pod by the apetalous line was attributed to reduced shading in the dense canopy during flowering, resulting in a 43% higher yield from the apetalous line.

Methods

B Line Trial

Eight spring canola lines were used in the trial (table 2.1), consisting of six apetalous B lines and two petalled lines used in commercial production of seed for crushing. Details of the line pedigrees are listed in table 2.1. The original apetalous line was discovered in the cross RU6/RU9, and so this ancestor is common to all apetalous lines used in these trials.

Table 2.1. Pedigrees of the B lines used in these trials.

Line	*Pedigree*
1803	Mutsu/3/Chikuzen//Zephyr/Bronowski/4/RU6/RU9
1806	BLN312//RU6/RU9
1809	BLN273//RU6/RU9
2804	RC6/3SVO2231//RU6/RU9
2807	Cyclone/3/SVO2231//RU6/RU9
2809	BLN341/SVO2231//RU6/RU9
20893	CHBL/3003//R/3*Maluka
30205	Oscar

A diverse range of genetic material was used in the production of the apetalous lines. The RU and RC material was originally developed by Agriculture Victoria, while the BLN lines are from the NSW Department of Agriculture. Maluka is an old Australian variety of which 30205 (Oscar) is a re-selection. Of the remaining lines SVO22331 is of Swedish origin, Cyclone is Canadian and CHBL is a Chinese line. The petalled lines used included 20893, which is used as the B line parent in the production of HYOLA 42, the only hybrid canola line currently available in Australia.

The target plant density was 100 plants/m^2 (MED), while two apetalous lines and one petalled line were also planted at HIGH (200 plants/m^2) and LOW (50 plants/m^2) densities (table 2.4).

The trials were directly sown with a ten-row precision cone seeder, with each plot being five metres long and consisting of ten rows spaced at 0.20 metres. Each plot contained a treatment comprising of a line x plant density variable, with the treatments randomised within three replicates. The replicates were arranged in a single strip with a two metre gap between replicates. The spring and autumn trials were sown in separate blocks and the agronomic details for the autumn and spring sowings are presented in table 2.2.

Destructive sampling in the autumn sown trial began on August 31, 1994, and was repeated every 2 weeks until maturity. For each sample an area of 0.25m^2 was harvested at ground level and used to determine leaf area and top dry weight.

An area of 2.1 m^2 was harvested by hand from all plots for yield determination. In the autumn trial 0.25 m^2 was harvested as a subsample to determine yield components. A subsample was not collected for the spring trial hence yield components were not available.

Results

Crop Growth: Autumn Sowing

The autumn sown trials were irrigated as required and did not experience any period of extended water stress during the trial. By June 10, 1994, some lines had begun to emerge and by June 16, 1994, all lines had emerged with a clear distinction in the time of emergence between the lines. Emergence was ranked by visual assessment, and was shown to be strongly related to seed weight, those lines with the heaviest seeds at sowing clearly showing the earliest and strongest emergence (table 2.3). One line, 1811 did not follow this trend, and despite having relatively heavy seed exhibited weak emergence.

Table 2.2. Agronomic procedures for 1994/95 trials, University Farm, Cambridge, Tasmania. Chemical rates are in grams of active ingredient/ha.

	Autumn Sowing	Spring Sowing/ Trial 2
Sowing Dates	30/5/94	13/10/94
Fertiliser Pre-drilled (N:P:K)	(9:14:17) 300 kg/ha	(9:14:17) 300 kg/ha
Weed Control Ground Preparation English couch (*Agropyron repens*)	Glyphosate 900 g/ha (16/5/94) Fluazifop 159 g/ha (8/7/94)	Glyphosate 900 g/ha (16/5/94)
Pest Control Red Legged-Earth Mites (*Halotydeus destructor*)	Omethoate 14.5 g/ha (27/7/94)	Omethoate 14.5 g/ha (30/10/94)
Bird Damage		Methiocarb 150 g/l (23/1/95)
Cabbage Aphids (*Brevicoryne brassicae*)		Pirimicarb 125 g/ha (23/1/95)
Final Harvest	15/12/94	8/2/95 13/2/95 (Trial 2)

Table 2.3. The strength of emergence of the different lines (ranked from strongest to weakest) compared to the weight of individual seeds at sowing.

Emergence	*Line*	*Seed Weight (mg)*
Strongest	*20893**	4.75
	2809	4.03
	2804	3.79
	*30205**	3.29
	1803	3.30
	2807	2.39
	1811	3.60
Weakest	*1806*	2.10

*petalled line

Table 2.4. The number of established plants for line and plant density treatments for the autumn trial 1994, calculated from two observations.

Line	*Target Plant Density*	*Plants/m^2*
1803	*100*	82.8
1806	*100*	66.1
1811	*100*	88.6
2804	*100*	72.3
2807	*100*	76.6
2809	*100*	83.2
*30205**	*100*	69.0
*20893**	*100*	78.8
1806 LOW	*50*	43.9
1811 LOW	*50*	42.1
30205 LOW*	*50*	34.1
1806 HIGH	*200*	212.8
1811 HIGH	*200*	169.9
30205 HIGH*	*200*	197.6

lsd(0.05)= 29.45
*petalled line

The number of established plants/m^2 was recorded on June 28, 1994 and July 28, 1994. The results presented in table 2.4 are the average of the two observations. There were no significant differences between the replicates or between different lines within the same planting density, with the exception of 1811 HIGH which produced significantly fewer plants/m^2 than 1806 HIGH. This indicated that generally the difference seen in the vigour of seedling emergence did not influence subsequent plant establishment.

Little vegetative growth occurred over the winter period from sowing until the first sample was taken at the end of August, 92 days after sowing (figure 2.1a). Through September rapid growth occurred in most lines as temperatures rose, with distinct differences between lines in terms of the rate of top dry matter (TDM) production. The two petalled lines 20893 and 30205 had the most rapid increase in TDM as temperatures began to rise and had achieved a high level of leaf cover 110 days after sowing (figure 2.1b). The apetalous lines in comparison generally showed a more gradual increase in TDM production, with lines 1803 and 2807 having especially low rates of accumulation. Line 2807 also failed to produce full leaf cover (Leaf Area Index, LAI >4), while 1803 managed to produce a good leaf cover despite having relatively low TDM production.

The yields obtained in this trial were low in comparison for an irrigated, autumn sown crop, due to a number of factors. In the autumn sown trial the plants reached a moderate size over the winter period, but did not receive any additional nitrogen fertiliser at the stem elongation stage. As a result any benefit associated with the apetalous character may have been lost, as the plants were unable to retain leaves due to low nitrogen levels. This was indicated by small leaf size and the development of red colouring in older leaves. The size of the plants at flowering in some treatments was also restricted which was shown to reduce the number of seeds produced per

pod. These trials also suffered extensive bird damage prior to and after windrowing, which also contributed to the relatively low yields.

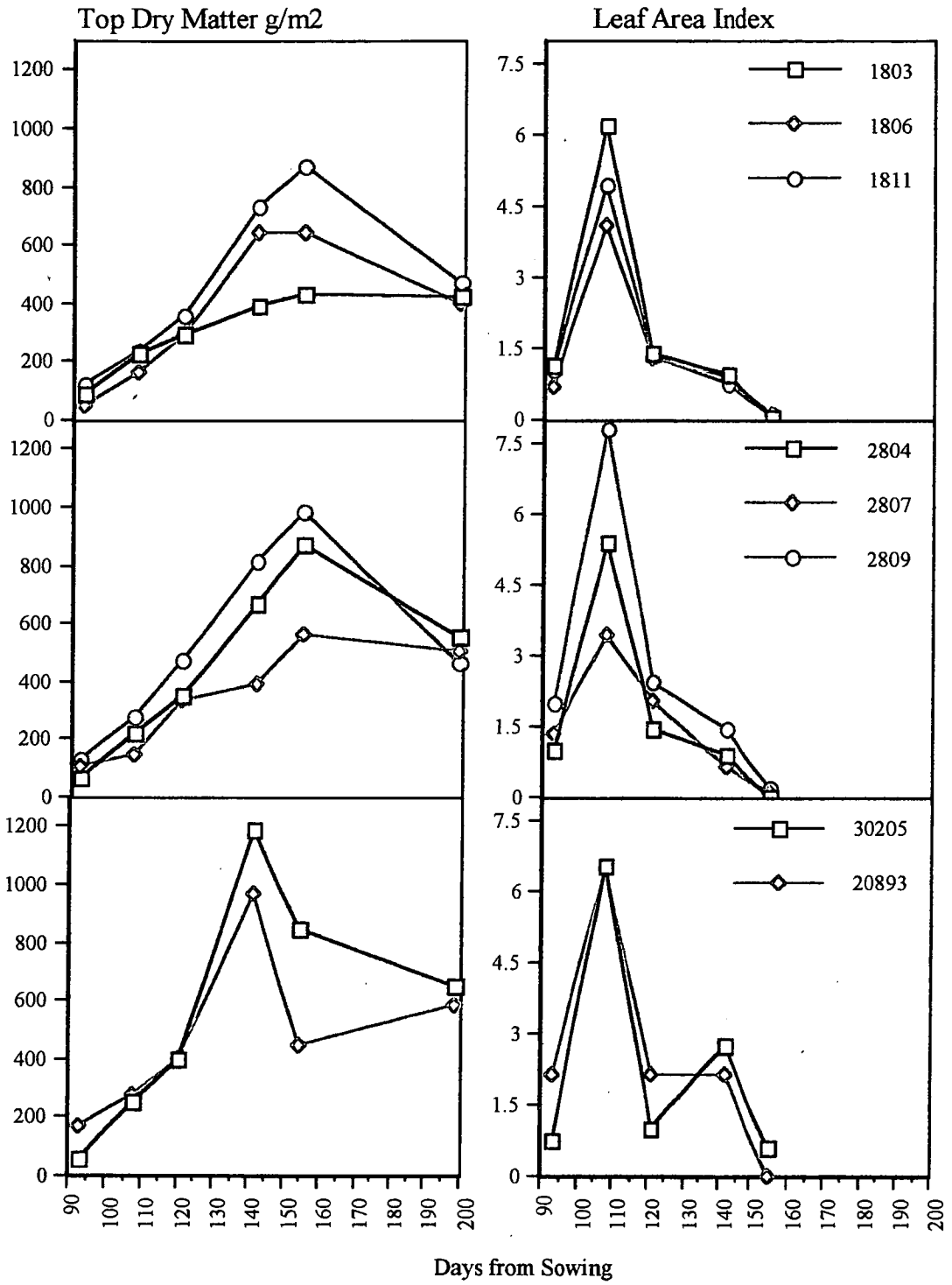


Figure (a) Figure (b)

Figure 2.1. (a) Top Dry Matter g/m^2 (TDM) production and (b) Leaf Area Index (LAI) of autumn sown trials 1994/95, for lines 1803, 1806, 1811, 2804, 2807, 2809, 30205 and 20893.

The poor vegetative growth of line 2807 had a marked effect on seed production (table 2.5), as it produced the lowest yield of all lines. The low seed yield of 2807 was chiefly due to a low number of seeds being retained per pod. Line 2807 also produced seeds with a low individual seed weight, which was surprising as plants producing a low number of seeds/pod usually compensate by producing heavier individual seeds. This line also produced a high number of aborted flowers or pods which may have used assimilate which could otherwise have been incorporated into developing seeds.

The best yielding lines produced twice the yield of line 2807, with lines 1803 and 20893 both producing over 1500 kg/ha. The relatively high yield produced by 1803 was surprising considering its low TDM, which may have also contributed to the low number of seeds/pod retained by this line. However the production of a high number of pods/m^2 (table 2.5), compensated for the few seeds/pod retained.

The yield components of the remaining lines were quite variable and could not be attributed to the apetalous character in this study. There was strong evidence of a compensatory effect between yield components, with lines such as 1811 and 2804 which produced a high number of productive pods retaining a low number of seeds/pod . The apetalous character did not appear to be directly responsible for the low number of seeds/pod produced by some lines as the apetalous line 2809 produced the highest number of seeds/pod of all the lines in this trial with 22 seeds/pod.

Lines 2807, 2804 and 1803 which produced the lowest number of seeds/pod, also produced the highest number of aborted pods. Whether the large number of unproductive pods competed for assimilate with developing seed or they were

produced in response to the low number of seeds/pod is not clear from this trial. However identifying the reason for the large number of unproductive pods would indicate if the low number of seeds/pod was a pollination or female fertility problem, or if it was caused by the competitive use of assimilates by unproductive parts of the plant.

Table 2.5. Seed yield and components of the autumn sowing 1994. Lines are ranked according to yield.

Lines	*Seed Yield kg/ha*	*Productive pods/m²*	*1000 seed wt. (g)*	*Seeds/Pod*	*Aborted pods/m²*
2807	707.8	1858	3.19	8.7	4032
2804	978.1	2380	3.86	10.7	1891
1806	986.2	1567	4.14	15.5	1195
30205	1115.5	1419	3.77	19.0	1001
1811	1162.8	2751	4.04	11.3	1312
2809	1428.1	1964	3.90	22.4	1526
1803	1517.1	3658	3.43	11.3	2814
20893	1542.8	2477	3.70	17.3	738
Plant density Treatment					
1806(LOW)	872.1	1781	3.93	12.3	1014
30205(LOW)	946.5	2052	3.46	14.8	751
1811(LOW)	1273.5	3963	4.33	7.4	1354
30205(HIGH)	1474.4	5744	3.50	9.4	2226
1811(HIGH)	1514.1	5158	4.12	7.0	2466
1806(HIGH)	1531.8	2151	3.93	15.3	2428
lsd(0.05)	*529.2*	*1239*	*0.18*	*7.4*	*1120*

Plant Density Treatments: Autumn Sowing

Increasing the number of plants/m^2 in the HIGH plant density treatment failed to produce higher levels of TDM in any of the lines (figure 2.2a), and in the case of the petalled line 30205 the HIGH treatment produced the lowest levels of TDM. The

differences between plant density treatments in TDM production for the apetalous lines were not as great and there did not appear to be any trends apparent between treatments.

The LAIs produced by different plant density treatments for line 1811 followed very similar trends (figure 2.2b), however there were noticeable differences between treatments for lines 1806 and the petalled line 30205, which warrant further discussion.

For line 1806 the LOW treatment reached the highest LAI level of the different plant densities in this particular line. A similar effect has been reported in other studies, (Rao *et al.*, 1991), where individual leaves expand to at least compensate for lower plant numbers. Line 1806 was then able to maintain a slightly higher LAI in the LOW treatment throughout the period when leaves were present, though this did not result in any yield benefits.

The apetalous character appeared to allow the retention of higher levels of LAI at MED and HIGH plant density treatments in comparison to the petalled variety 30205. After peak LAIs were reached the MED and HIGH treatments had a more rapid decline in LAI than the LOW treatment for line 30205 (figure 2.2b).

Generally the HIGH treatments produced significantly higher yields (table 2.5) than the LOW treatments, with the MED treatment yields being intermediate. An exception to this general trend was seen in the LOW treatment of 1811, which yielded significantly more than the LOW treatments of 1806, and 30205. The LOW treatment of 1811 produced a high number of productive pods with relatively few but heavy seeds whereas the LOW treatments of 1806 and 30205 produced more seeds/pod but with significantly less productive pods/m^2, hence yields were lower.

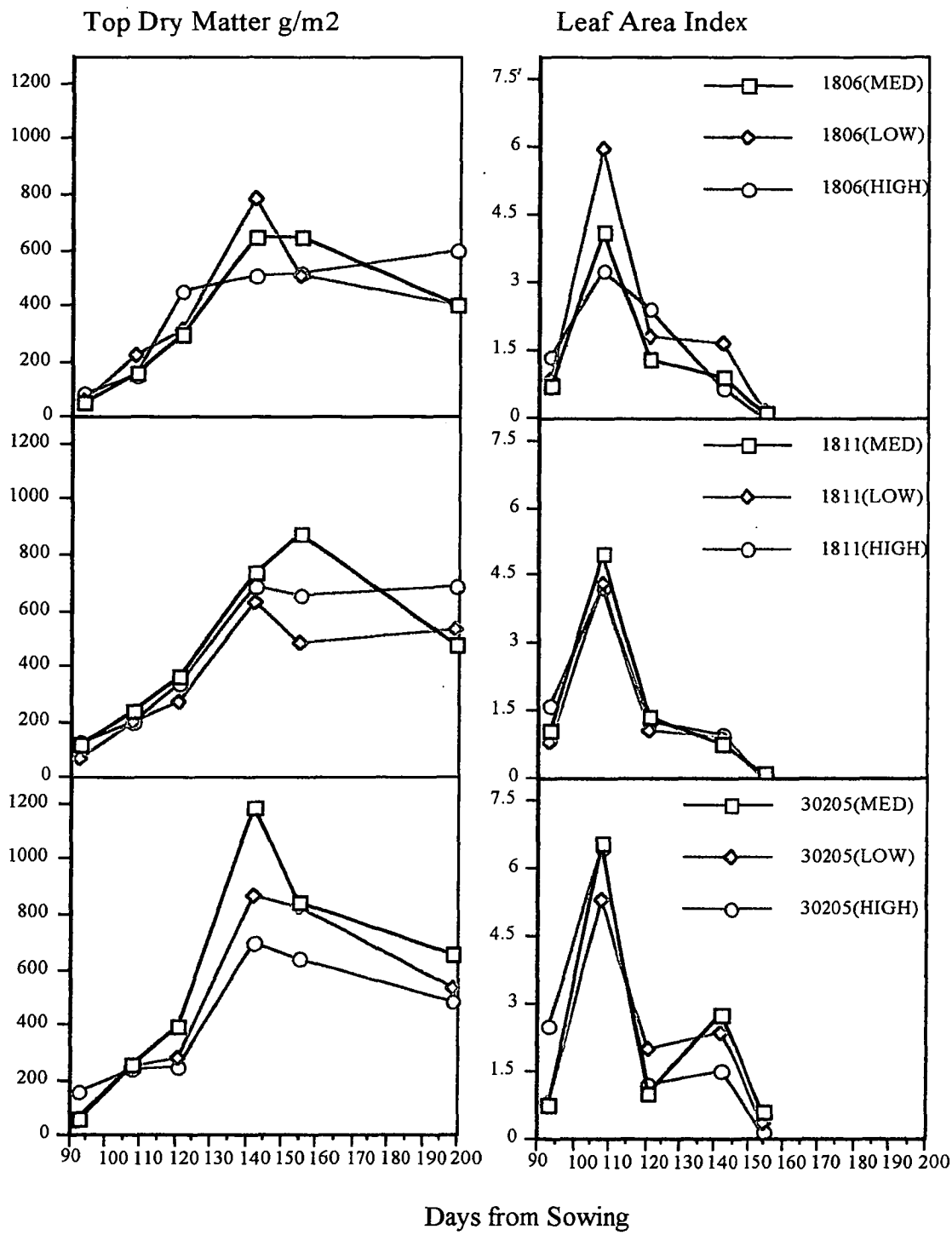


Figure 2.2. (a) Top Dry Matter g/m^2 (TDM) production and (b) Leaf Area Index (LAI) of lines subjected to plant density treatments from the autumn sown trials 1994/95.

The ability of the canola plant to compensate for limiting yield components was evident in some of the plant density treatments, which also indicated a possible benefit conferred by the apetalous characteristic.

Line 30205 produced a large number of productive pods/m^2 in the HIGH plant density treatment, while retaining fewer seeds/pod in comparison with the MED and LOW treatments. In contrast line 1806 retained a similar number of seeds/pod in all treatments. Therefore despite line 1806 producing significantly fewer productive pods than lines 1811 and 30205 in the HIGH plant density treatment, a relatively high yield was still possible.

The ability of line 1806 to retain more seeds/pod at the HIGH plant density treatment could be attributed to better light penetration through the apetalous flower canopy. This result was not replicated for line 1811, however the fact that this line retained only a low number of seeds/pod even in the LOW plant density treatment suggests that some other influence was restricting this yield component.

In the case of the plant density trial the lines and treatments that produced circa 2000 productive pods/m^2 were able to retain at least 12 seeds/pod (table 2.4), while the treatments producing more pods had low seeds/pod numbers. The lines with low seeds/pod numbers also had low levels of TDM at flowering which reduced their ability to retain seeds (figure 2.3), while the later flowering of 1806 allowed all treatments to reach higher levels of TDM before flowering. Although low numbers of seeds/pod did not result in significantly lower yields in this trial, it would be more influential under less limiting conditions.

The ability of a line to retain a high number of seeds/pod was strongly influenced by the amount of TDM produced by the time flowering had begun. The polynomial second order regression analysis (figure 2.3) of seeds/pod and TDM/m^2 had a highly significant R^2 value of 0.69 ($P<0.001$) which accounted for much of the variation in the numbers of seeds/pod produced by the various lines. While the retention of a high number of seeds/pod has been reported as an important character in determining yield in other studies (Mendham *et al.*, 1981; Mendham *et al.*, 1984) it was not as critical in this trial. High numbers of productive pods/m^2 were able to compensate for low seed retention, as illustrated by relatively high yield obtained from 1803.

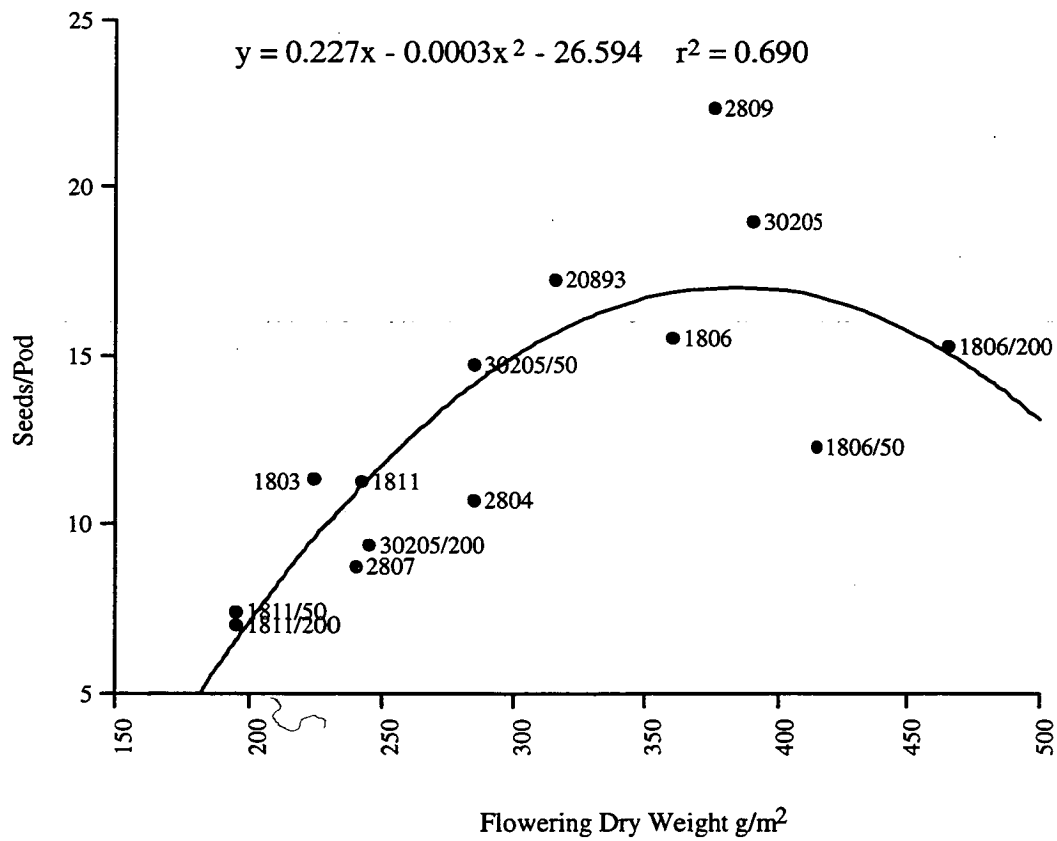


Figure 2.3. The relationship between top dry matter at flowering and the number of seeds retained per pod, for autumn sown trials 1994/95.

Crop Growth: Spring Sowing

A second sowing was conducted in spring to determine the effect of delayed sowing on yield. The conditions experienced during this trial made plant growth difficult, as during establishment and flowering hot, dry weather made it difficult to avoid water stress. The plots were heavily infested with red-legged earth mites (*Halotydeus destructor*) which caused extensive damage to the young seedlings retarding TDM accumulation and preventing all lines from achieving full leaf cover.

Because of these setbacks, and a shorter period for vegetative growth and seed development, all lines produced lower yields in the spring sowing (table 2.6). Line 30205 in the MED plant density treatment was the only line not to have a large difference between the two sowing dates, however yields from the HIGH and LOW plant density treatments for this line had lower yields in the spring sowing. This tends to indicate that the lack of response was due to experimental error and not a line effect.

With the exception of 30205 most lines had at least a 45% reduction in yield which appeared to be the result of low levels of TDM accumulation caused by poor establishment and water stress. This restricted the plant's ability to provide an adequate framework for potential pod sites and then fill the seed after fertilisation. Without yield component data it is difficult to be more specific in explaining the reasons for the differences in yield.

Table 2.6. Yields of the spring sown trial 1994/95. Yields of the spring sowing are also given as a percentage of the autumn yields.

Line	*Yield kg/ha*	
2807	287.9	41%
2804	469.5	48%
1806	536.1	54%
30205	986.8	88%
1811	545.0	47%
2809	547.7	40%
1803	441.0	29%
20893	841.2	55%
Plant Density Treatments		
1806(LOW)	239.4	27%
30205(LOW)	252.0	27%
1811(LOW)	716.9	56%
30205(HIGH)	613.6	42%
1811(HIGH)	702.4	46%
1806(HIGH)	659.8	43%
lsd (0.05)=481.4		

The HIGH and MED plant density treatments of lines 1806 and 1811 had similar reductions in yield between sowing dates. The LOW treatments of 1806 and 30205 had larger differences between sowing dates. This was probably caused by the shorter growing season in spring restricting the opportunity for yield compensation at low plant density. Treatment 1811 LOW was not as affected and produced significantly higher yields than the other lines for this plant density.

Discussion

Previous trials conducted with autumn-sown irrigated canola in Tasmania have produced seed yields over 5.5 t/ha (Mendham et al., 1984; Mendham et al., 1990),

which approached the theoretical maximum yield for rapeseed proposed by Habekotte (1993) of 5.8 t/ha.

The yields obtained in this trial were low in comparison for an irrigated, autumn sown crop, due to a number of factors. In the autumn sown trial the plants reached a moderate size over the winter period, but did not receive any additional nitrogen fertiliser at the stem elongation stage. As a result any benefit associated with the apetalous character may have been lost, as the plants were unable to retain leaves due to low nitrogen levels. This was indicated by small leaf size and the development of red colouring in older leaves. The size of the plants at flowering in some treatments was also restricted which was shown to reduce the number of seeds produced per pod. These trials also suffered extensive bird damage prior to and after windrowing, which also contributed to the relatively low yields.

The yields of the spring sowing showed a similar reduction compared with autumn sown crops as described by Mendham *et al.* (1990) for canola in southern Tasmania. Both trials were sown in mid- to late-October and produced yields of less than 1 t/ha. While the yield components of the spring sowing were not available for this trial, Mendham *et al.* (1990) described how the reduction in yield was the result of a reduction in the number of productive pods produced.

The apetalous character did not result in a distinct yield advantage in these trials, contrary to the results produced by Rao *et al.* (1991). However even if yield differences between the apetalous and petalled lines were detected, it would not be possible to attribute this solely to the flowering characteristic of the lines investigated, due to the other genetic differences between them.

There was some indication that the apetalous character may have potential benefits in crops that produce heavy crop canopies, such as occurs with high plant densities.

The apetalous line 1806 retained a similar number of seeds/pod in all plant density treatments, while the petalled line 30205 produced fewer seeds/pod in the HIGH plant density treatment. This may have been the result of better light infiltration into the apetalous flower canopy, though the lack of different LAI levels in the respective treatment did not support this. The apetalous line 1811, which was also used in the plant density treatments, produced low numbers of seeds/pod in both high and low density treatments. This illustrated that the apetalous character is only of potential benefit in lines which are able to produce a high number of seeds/pod under non-radiation limiting conditions.

Despite the questions raised by the genetic differences existing between the apetalous and petalled lines, the results produced by Rao *et al.* (1991) and Fray *et al.* (1996) indicated that the apetalous character would be of benefit in high biomass, heavily flowering canola crops. The fact that such differences were not shown conclusively in these trials was probably more a reflection on crop management than any other factor. In crops grown without limitations, which can express their full genetic yield potential, it is highly likely that yield differences would be apparent between the apetalous and petalled line.

This preliminary trial did demonstrate that there was quite large variation in the agronomic qualities of the apetalous lines used, and that the best of the apetalous lines had the ability to produce yields at least similar to the highest yielding petalled lines in the conditions under which the trials were conducted.

It would also be a reasonable assumption that the F_1 hybrids produced from the respective A lines of the B lines used in this trial, would produce higher yields under less limiting conditions with the expected heterosis effect.

The results from this trial established a benchmark for yield of the parent lines used to produce the F_1 hybrid. While the apetalous lines did not out-perform the petalled lines, they were at least comparable indicating that the respective A lines should be capable of producing economic yields of hybrid seed.

Trial II

Hybrid Seed Production

Objective

This trial was conducted to give an indication of what hybrid seed yields could be expected from apetalous A lines (male sterile). A petalled A line was used in these trials to act as a yield benchmark from a conventional line under the prevailing conditions. The yields produced from the B lines in Trial 1 indicated that some apetalous lines had the ability to produce similar yields to the highest yielding petalled variety. This supported the results of Rao *et al.* (1988) that the apetalous character was at least not limiting to yield in male fertile lines.

A further intention of this trial was to provide information to allow the selection of several apetalous lines to be used in future trials for more detailed investigation of the factors influencing yield. Yields of A line plants depend not only on the agronomic characteristics of a given variety, but also the successful transfer of pollen from the pollen source. Ideally in such a study isogenic lines differing in only the apetalous character should be used so that other genetic influences on yield can be eliminated. Although such lines were not available, the results of the B line trial did indicate that some of the apetalous lines had similar agronomic potential to the petalled line.

As *Ogura* restorer lines were not available at this stage, the B lines of each A

line were used as pollen sources. This ensured the synchrony of flowering that is essential for maximum pollination of the A line plants.

Methods

In this trial the apetalous A lines of the B lines used in Trial I were used (table 2.7, page 55). The petalled line included in this trial, 20894, is genetically identical to line 20893 apart from containing the same *Ogura* cytoplasm as in the apetalous A lines. Any differences in performance could then be attributed to the apetalous character or genetic differences between lines and not the *Ogura* cytoplasm.

Ideally, when investigating hybrid seed production, lines should be isolated to avoid pollen transfer between plots. Due to the number of lines included in the trial and the requirement of a randomised block design for statistical purposes, isolation by distance was not considered to be practical.

Therefore in order to minimise pollen transfer between lines, plots were sown parallel to each other to maximise the distance between B lines of adjacent plots. The trial was sown on October 13, 1994 with a ten row precision cone seeder with a target plant density of 100 plants/m^2. Each plot consisted of 20 rows of A line plants, (constituting a subplot) with 0.20 metres between rows, on either side of four rows of B line plants with a 0.30 metre space between the B line and A line plots. Seed production plots for each line were replicated twice in this trial. Each plot was 20 metres long and only the middle eight metres of each subplot was harvested to minimise the effect of pollination from other plots. The same agronomic procedures were followed as for the B line spring sowing trial (trial 1) and are presented in table 2.2.

Destructive sampling of a 0.25 m^2 area of each plot began on November 18, 1994 and was repeated every two weeks until January 13, 1995. From these samples LAI and above-ground dry weights were determined, and the number of flowers, pods and missing pods was calculated from a subsample of six plants.

To determine final yield the outer A line row of each plot was discarded and the middle eight metres of each subplot was cut at ground level by hand for drying and threshing. The total area harvested was 30.40 m^2 for each subplot, with analysis of variance used to determine the significance of differences between lines for yield.

Results

Crop Growth

During the trial, difficult conditions for plant growth were experienced. These consisted of high levels of temperature and water stress, which combined with a severe infestation of red-legged earth mites (*Halotydeus destructor*). This resulted in no lines showing dramatic increases in TDM until the second destructive sample, which was taken 47 days after sowing (figure 2.4a). The plots were slow to establish leaf cover with no lines managing to achieve full coverage (LAI all less than 4, figure 2.4b).

There did not appear to be any major differences between the lines in the rate of TDM accumulation. Lines 1803, 1811 and 2807 did show a reduction in TDM at the last sample date, but it is unclear why this occurred. For lines 1811 and 2807 this was related to very low yield and could be explained by the senescence of

unproductive pods. However line 1803 produced a significantly higher yield than both of these lines.

There did appear to be differences in leaf production both between the apetalous and petalled lines, and within the apetalous lines that were related to pod production and final yield. The petalled line, 20894, reached a peak LAI 47 days after sowing which slowly declined over the rest of the sampling period. The highest yielding apetalous lines 1803, 1806 and 2804 followed a similar pattern of an early peak LAI followed by a steady decline. For line 1806 the peak LAI was two weeks later, due to it being a later flowering line.

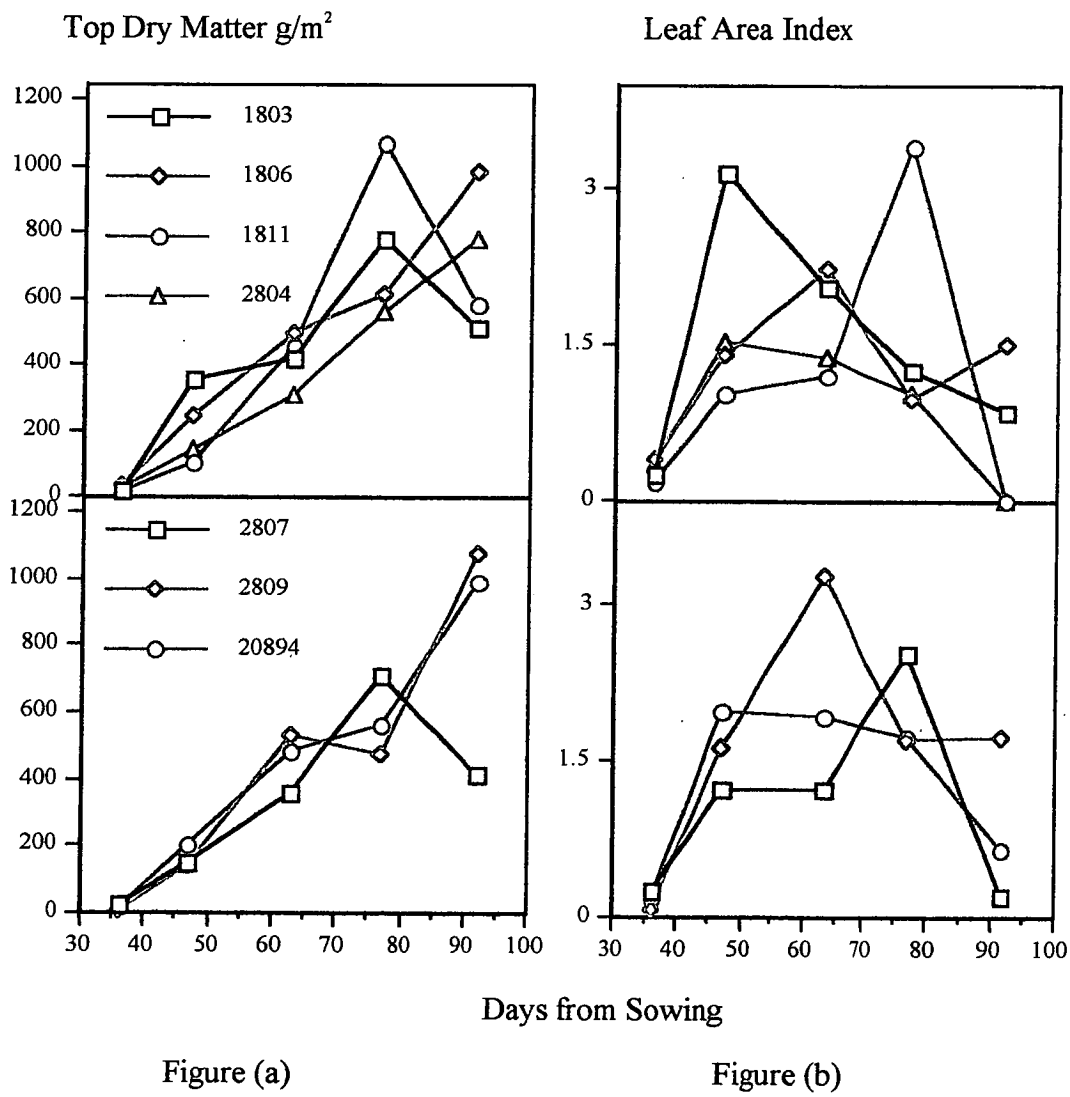


Figure 2.4. (a) Top Dry Matter g/m^2 (TDM) production and (b) Leaf Area Index (LAI) of spring sown hybrid trial 1994/95.

In contrast, lines 1811, 2807 and 2809 produced higher peak LAI's later in the sampling period, and then showed a rapid decline. Lines 1811 and 2807 also produced the lowest number of productive pods/plant (Figure 2.5), among the apetalous lines, as well as very low yields.

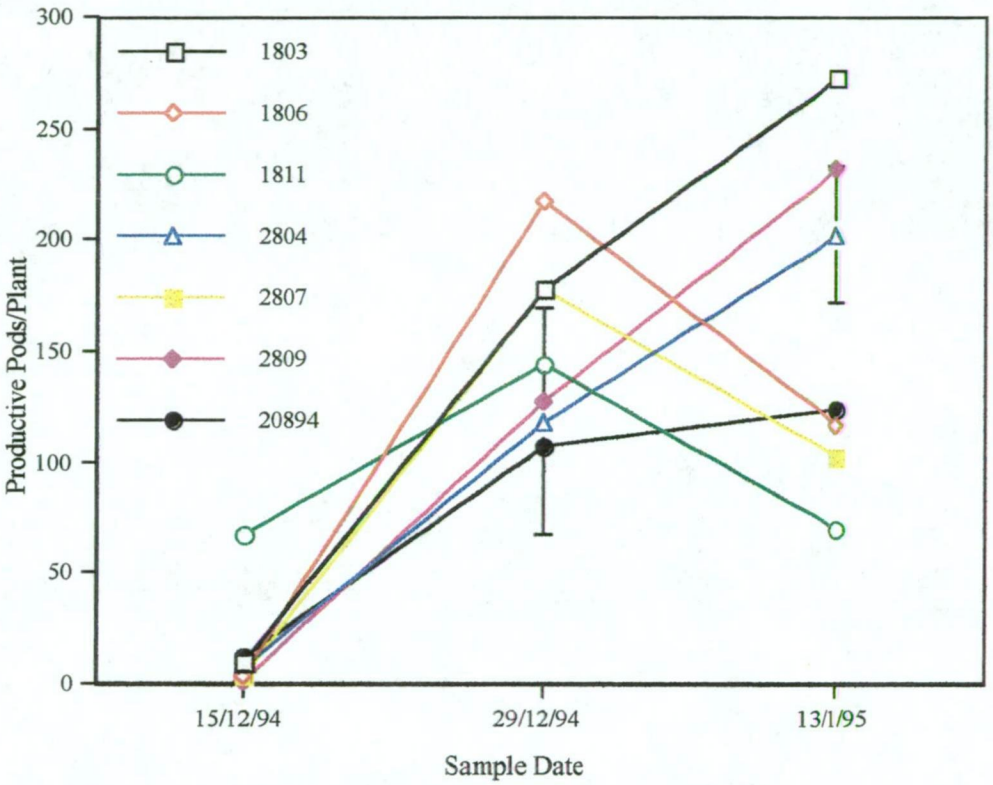


Figure 2.5. Productive pods/plant calculated from destructive samples of the spring sown hybrid trial. Bars represent S.E. for each observation.

In terms of total seed yield, the petalled line 20894 produced a respectable 1500 kg/ha, almost three times higher than the highest yielding apetalous line 1806 (table 2.7). Lines 1806 and 1803, were the only apetalous lines that showed some promise, yielding around 500 kg/ha. The remaining apetalous lines had very low yields with only line 2804 yielding more than 200 kg/ha.

There did not appear to be any major differences in the rate of TDM accumulation that could offer any explanation for yield differences. Although

yield component data were not collected at final harvest for this trial, the available information suggested that the low yield of the apetalous plants was associated with low seed set. The apetalous lines produced very high individual seed weights, which is usually the result of a plant not approaching its potential seed set. Thus a greater proportion of assimilates is available to individual seeds.

From destructive sample data (figure 2.5) the apetalous lines that produced most productive pods went on to produce significantly higher yields than the other apetalous lines. However even with more productive pods than the petalled line 20894, lines 1803, 1806 and 2804 still had significantly lower yields. For these lines the results indicate that pod production did not restrict yield and supports the theory that the number of seeds/pod was the main limiting factor. The remaining apetalous lines had fewer productive pods, which also greatly restricted final yield.

Table 2.7. Yields and 1000 seed weights of 1994/95 spring sown A and B lines.

Lines	*A Line Yield kg/ha*	*B Line Yield kg/ha*	*1000 Seed Wt. gms*
1803	424	441	6.01
1806	587	536	4.75
1811	62	545	6.00
2804	247	469	4.98
2807	97	287	4.97
2809	79	547	5.84
20894	1473	841	3.83
	lsd(0.05)=320.0		*lsd(0.05)=0.25*

While statistical comparisons can not be made between the yields in Trial 2 and B lines yield of Trial 1, they were sown on the same day and received the same management, so considering that they are genetically identical (apart from the CMS factor), they should have the same genetic yield potential if adequately pollinated.

The A lines of 1803, 1806 and to some extent 2804 produced comparable yields to their B line counterparts (table 2.7). 20894 had a large yield increase which is difficult to explain, but it may have been due to seed losses in the B line trials due to shattering and bird damage.

The very poor performance of 1811, 2807 and 2809 raise the most questions in this comparison. As previously discussed poor vegetative growth would not appear to be the cause of the low yield or indeed low flower numbers.

In these particular lines, the combination of the apetalous character and male sterility was extremely detrimental to yield under the conditions experienced in this trial. The fact that lines 1803 and 1806 were not affected to the same degree suggests that this is due to genetic differences between the lines, and is not specifically a function of the *Ogura* CMS or apetalous character.

Without yield component data is it not possible to ascertain the reason for the poor performance of these lines. The remaining chapters will hopefully shed more light on the problem, and attempt to explain why such large differences existed between lines containing outwardly very similar characteristics.

Discussion

The climatic conditions experienced during this trial were not typical for the area, and provided an extreme environment in which potential differences

between lines would be minimised. Table 2.8 contains the weather data for the flowering period of this trial, (December, 1994) compared with the long term average for the same period. The temperatures experienced were much higher than normal as indicated by the average daily maximum temperature being 3.9°C higher in 1994 and there being 6 days in which the daily maximum temperature exceeded 30°C. The high temperatures combined with very little rainfall and high evaporation rates produced very difficult conditions for plant growth.

Table 2.8. Long Term (1958-1996) and 1994 December weather data for Hobart Airport. *(Bureau of Meteorology, Australia)*

	Averages for December	
	Long term	*1994*
Daily temperature max °C	20.4	24.3
Daily temperature min °C	10.6	11.2
Days > 30 °C	1.1	6
Monthly rainfall mm	58.9	0.8
Monthly evaporation mm	182.9	261.2

Despite some lines showing a reduction in TDM at the final sampling date, which could not be satisfactorily explained, growth rates were similar for all lines. The conditions experienced during the trial did not appear to have any differential effects on plant growth between the lines.

However, it is clear that some factor had a significant effect on the final yield of the A line plants. Results obtained in Trial 1 did not appear to show such an influence on the apetalous B lines. As previously mentioned this factor did not appear to be related to differences in vegetative growth, as there were no obvious differences in biomass accumulation between the A lines. It was also clear that flower production was not yield-limiting, for from the destructive samples data all apetalous lines

produced at least as many, and in some lines significantly more, pods than the petalled variety.

There were however clear differences between lines in the number of pods which were retained and that produced seed. From the available data, this was one of the main determinants of final yield in the apetalous A lines. The relatively high yields produced by lines 1806 and 20894 from a lower number of productive pods than 1803 and 2804 suggest that the former lines produced more seeds/pod and demonstrate a possible compensatory flower production effect occurring in the remaining apetalous lines. The ability of line 1806 to possibly retain more seeds/pod may be related to its later flowering period, though it is not clear from this trial.

All lines in this trial contained *Ogura* cytoplasm, and the acceptable performance of the petalled line 20894 containing this cytoplasm would suggest that the low yields of the apetalous lines could not be attributed to this factor. However, the B line spring sowing (Trial 1) indicated that some apetalous lines were capable of producing similar yields to the petalled varieties.

While agronomic differences have not been considered at this stage, the large difference in yield between petalled A line and apetalous A line, which did not exist in the B line comparisons in Trial 1, would suggest that the apetalous/male sterility interaction is likely to be having a greater influence on seed yield than genetic potential.

While this trial did not establish why the apetalous A lines were not able to perform as well as the petalled A line, it did indicate that the problem involved pod and seed set, and hence pollen transfer. The following trials were designed to investigate in more detail the development of yield components, and their comparative influence on final yield between different lines.

Chapter 3.

Hybrid Seed Production Trials 1995/96

Objectives

The trials conducted in 1995/96 were designed to identify the reasons for the difference in seed yield between selected apetalous A lines and the petalled A line used in the 1994/95 season. The objective was to determine if yield differences between the petalled and apetalous lines could be explained by the combination of the apetalous character and male sterility, or if it was due to differences in agronomic characteristics between the lines. The two highest yielding apetalous lines, 1803 and 1806, were chosen as well as the low yielding line 2807 for comparison. The petalled A line 20894 was again used as a benchmark, being derived from a proven commercial variety and also containing the *Ogura* cytoplasm.

To examine what effect climatic conditions at flowering had on seed set and subsequent yield two sowing dates were chosen. The first sowing was planned for late September so the plants would be flowering in November, and the second sowing was planned for November for flowering in January. The anticipated flowering times were based on the long-term climatic averages for the area. The climatic data indicate that the mean daily maximum temperature is higher in January compared with November, and the likelihood of experiencing temperatures higher than 30°C is greater.

Introduction

The significant correlation between seeds/pod and total dry matter at flowering established in the previous chapter for the autumn sown B line trial suggests that seed set is mainly determined by the ability of a crop to produce assimilate during flowering. However this would only be expected when pollination and subsequent fertilisation and pod density do not limit seed number. This is generally the case in self-fertile plants under field conditions (Habekotte, 1993). Results from the previous seasons' trials indicate that seed set may have had a major influence on the yield of apetalous A lines (male sterile).

Canola has the ability to produce flowers on secondary and tertiary branches, however 75% of pods that are retained to harvest have been shown to develop from flowers which open within 11 days of the first flower opening (Tayo and Morgan, 1975). Therefore the period of 1-2 weeks around first flower are critical in determining yield, and any stress placed on the plants during this period, such as high temperatures, will result in yield reduction (Morrison, 1993).

Morrison (1993) examined the effect of high temperature on male and female fertility of two varieties of summer rape. Plants were grown in hot (27:17°C) and cool (22:15°C) growth cabinets and moved to different temperature regimes at various stages of development. The results showed that plants grown to flowering in the hot cabinet were almost entirely sterile and the most sensitive stage to heat stress was from late bud to early seed development.

Heat stress was found to affect both the male and female reproductive organs, although it appeared female fertility was affected to a greater extent. The results indicated that while heat stressed pollen was fertile it had reduced vigour. Heat stressed stigmas pollinated with pollen from either hot or cool growth cabinets produced significantly less productive pods, lower seed weights and less seeds/pod

than stigmas from the cold cabinet. The combination of heat stressed stigmas and pollen produced the least number of seeds/pod. There were significant differences between the two cultivars tested indicating that there may be genetic variability for heat stress sensitivity (Morrison, 1993).

Reduced numbers of productive pods and seeds/pod effectively reduces the sink capacity of the plant. Tommey and Evans (1992) investigated the effect of a reduction in the sink capacity on compensatory growth of winter oilseed rape. These experiments more closely imitated poor seed set than other plant manipulation experiments as the stigma, style and anthers of individual flowers were removed rather than entire branches and flowers (Tommey and Evans, 1992). As petals were left intact the natural light profile within the plant canopy was maintained.

It was found that the number of branches and the total number of flowers produced increased significantly when flowers were removed from the main stem and uppermost branches. This compensatory growth by the plant and the production of more potential pods was unable to compensate in terms of yield for the loss of the upper branches. In contrast the removal of flowers from lower branches was offset by an increase in the productivity of the higher order branches and an increase in overall yield.

Methods

Lines 1803, 1806, 2807 and 20894 were used in the trials, sown with a six row air seeder which was calibrated for a target density of 100 plants/m^2, with rows spaced at 0.20 metres. Each plot consisted of twelve rows of A line (subplot), planted on either side of four rows of B line constituting 28 rows in total. Each plot was 15 metres long and randomly replicated within two blocks. Within blocks, plots were

sown linearly and blocks were positioned side by side with rows running parallel in respective blocks. In the area between plots and blocks, A line plants were used as 'fillers' to reduce pollen transfer between lines.

Fertiliser application levels were determined after a soil test (table 3.2) indicated low levels of sulfur. Canola crops require a relatively large amount of sulfur for the manufacture of both proteins and glucosinolates. Considering this and the use of high analysis fertilisers which contain very low levels of sulfur it is important to provide adequate levels in the applied fertiliser. The level of nutrients applied to the trials and agronomic details are presented in table 3.1.

On October 17, 1995 a combination of high temperatures and winds gusting up to 120 kph severely damaged large parts of the plots of trial 1. The areas which survived were those at the bottom of the slope on heavier ground. The sections of plots which survived were sampled and harvested. Due to the earlier damage the results for the yield components were incomplete. The trial was resown as Trial 2 on November 14, 1995, sowing being delayed until this date due to several weeks of hot weather. The planned second sowing, which effectively became a third sowing (trial 3), was then delayed until December.

Trial 3 also suffered problems in plant establishment due to sowing difficulties, which resulted in low plant densities and weed problems. Consequently, only two lines were retained until harvest.

Table 3.1. Agronomic procedures for 1995/96 trials, University Farm, Cambridge, Tasmania. Chemical rates are in grams of active ingredient/ha.

	Trial 1	Trial 2	Trial 3
Sowing date	20/9/95	14/11/95	15/12/95
Fertiliser			
Pre-drilled	N : 49.5 kg/ha P : 40.0 kg/ha K : 75.0 kg/ha S : 39.0 kg/ha	N : 49.0 kg/ha P : 20 kg/ha K : 25 kg/ha S : 33.5 kg/ha	N : 49.0 kg/ha P : 20 kg/ha K : 25 kg/ha S : 33.5 kg/ha
Top dressing	N : 90 kg/ha (28/11/95)	N : 50 kg/ha (30/11/95)	N : 50 kg/ha (29/1/96)
Weed Control			
Trifluralin	600 g/ha (13/9/95)	600 g/ha (7/11/95)	600 g/ha (13/12/95)
Pest control			
Redlegged earth mites (*Halotydeus destructor*)	Omethoate 14.5 g/ha (2/10/95)	Omethoate 14.5 g/ha (22/11/95)	
Bird Damage	Methiocarb 150 g/ha (2/2/96)	Methiocarb 150 g/ha (4/12/95)	
Aphids (*Brevicoryne brassicae*)		Pirimicarb 125 g/ha (22/1/95)	
Cabbage moth/Heliothis (*Plutella xylostella*)		Chlorpyrifos 600 g/ha (22/1/95) Permethrin 75 g/ha (7/2/96)	Permethrin 75 g/ha (7/2/96)
Final Harvest	Line 1803 (20/2/96) 1806 (20/2/96) 20894 (13/2/96)	Line 1803 (24/4/96) 1806 (23/4/96) 2807 (24/4/96) 20894 (20/3/96)	Line 1806 (3/5/96) 20894 (2/5/96)

Table 3.2. Soil test results for 1995/96 trials, nutrient levels in ppm.

LOI%	*pH*	*P*	*K*	*Ca*	*Mg*	*SO_4*	*Mn*	*Zn*	*Cu*	*B*
4.1	6.2	9	36	1120	230	<5	8	2.2	1.0	0.9

(LOI= loss on ignition)

Loktronic™ tensiometers were placed in blocks to assist in irrigation management so plants did not experience any periods of severe water stress. All plots were top dressed with nitrogen by hand at the beginning of stem elongation (table 3.1). Destructive samples of $0.25m^2$ were taken weekly in trial 2 to estimate TDM and

LAI, and a subsample of five plants was further analysed to determine flower, pod and aborted pod numbers. For the final harvest the outer-most row was discarded and a five metre section from the middle of each row was cut by hand, labelled and bundled together for drying. Bundles were threshed individually with a mechanical plot thresher allowing yield and 1000 seed weight to be calculated on a row basis. This data will be discussed in chapter 6. Total yield was calculated from the summation of individual row yields, which constituted 11.0 m^2 per subplot in total. Bundles from later sowings were also harvest row by row, but wet weather experienced late in the season made it necessary to transfer the bundles to a shed for drying. A subsample of five plants from the row closest to the pollen source and then every second row was collected to estimate yield components.

Results

The most complete set of results was obtained from trial 2, which will be examined first, before brief consideration of trials 1 and 3.

Crop Yield

Trial 2

The petalled line 20894 produced a yield of almost 3000 kg/ha (table 3.3), which was very high for hybrid seed production under Tasmanian conditions. This was significantly higher than the apetalous lines which all produced similar yields. The yield component which appeared to be chiefly responsible for the low yield of the apetalous lines was the number of seeds produced per pod, this being significantly lower in all the apetalous lines compared with 20894. In contrast, the number of productive pods produced per square metre was similar in all lines, while the number of aborted pods/m^2 was much higher in the apetalous lines. Overall, 20894

produced seed-containing pods from 60% of potential pods, while the apetalous lines converted around 20% of potential pods into productive pods.

As the apetalous lines had less seeds as assimilate sinks than 20894, more resources were available to individual seeds during seed fill, resulting in significantly higher individual seed weights. However, it is clear that the seed sink produced by the apetalous lines was insufficient, as heavier individual seed weights were unable to compensate for poorer pod and seed set.

Table 3.3. Yield and yield components for all 1995/96 trials.

			Pod Numbers/m²		
	Yield kg/ha	*1000 Seed Wt. g*	*Productive*	*Aborted*	*Seeds/pod*
Trial 1					
1803	1321.3	4.72	7960*	7350*	3.5*
1806	1642.8	4.51	5013*	4511*	7.3*
20894	3403.1	4.03	5408*	1360*	14.9*
lsd(0.05)	*562.0*	*0.24*			
Trial 2					
1803	837.3	6.58	4347	16817	3.1
1806	872.7	5.98	3571	11600	5.1
2807	863.5	5.38	4554	16801	(4.0)*
20894	2996.7	4.34	3477	2252	19.9
lsd(0.05)	*480.1*	*0.39*	*NS*	*3933*	*1.39*
Trial 3					
1806	2243.6	4.58	7470*	7728*	8.8*
20894	3529.3	3.65	6479*	2359*	16.7*
lsd(0.05)	*838.7*	*0.15*			

*results incomplete, data estimated from final destructive sample.

Crop Growth and Flowering

Trial 2

The low yields of the apetalous lines did not appear to be the result of restricted vegetative growth, as demonstrated by Figure 3.1. All lines showed similar levels of TDM production up to the sample taken on the 7/2/96, when there was a clear divergence between the lines in growth rates. The apetalous lines 1803 and 1806 both showed very little increase in TDM production after this stage, while lines 2807 and 20894 both showed the most rapid increase in TDM for the entire growth period after this point.

In the case of the petalled line 20894, this was the period of seed fill, and the heavy seed load produced by this line resulted in the rapid increase of TDM. The same was obviously not so for line 2807, and it appears that the period of rapid growth exhibited by this line was associated with continued vegetative growth.

In the previous chapter, the relationship between plant size at flowering and the number of seeds retained per pod for male fertile plants was clearly demonstrated (figure 2.3). In this situation the apetalous lines attained at least the same size as the petalled line by peak flowering, and even more in the case of line 1806, the later flowering variety. Therefore, the low yields of the apetalous lines could not be attributed to the lack of vegetative growth.

The point of peak flowering had a distinct effect on the LAI of the petalled line 20894, while the effect on the apetalous lines was much less pronounced. Once peak flowering was reached by line 20894, a rapid loss in LAI followed (figure 3.2). By the time flowering had practically finished (figure 3.3), on the 30/1/96, the loss of LAI ceased and 20894 was able to maintain some foliage through to the penultimate sample, mainly as small leaves subtending branches.

Figure 3.1. Top dry matter accumulation of trial 2. ▼ Indicates peak flowering

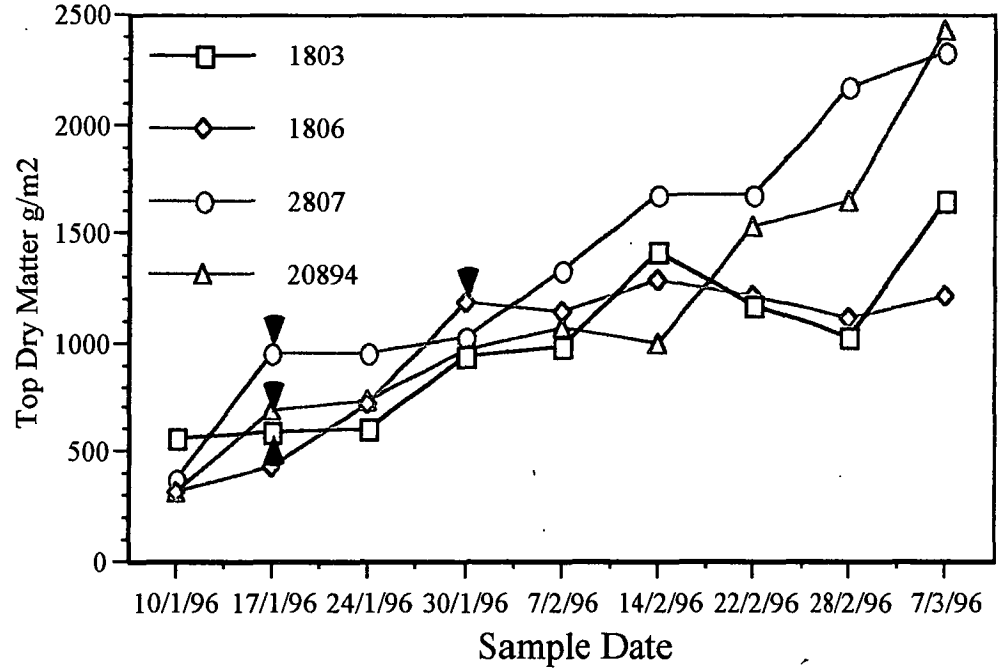
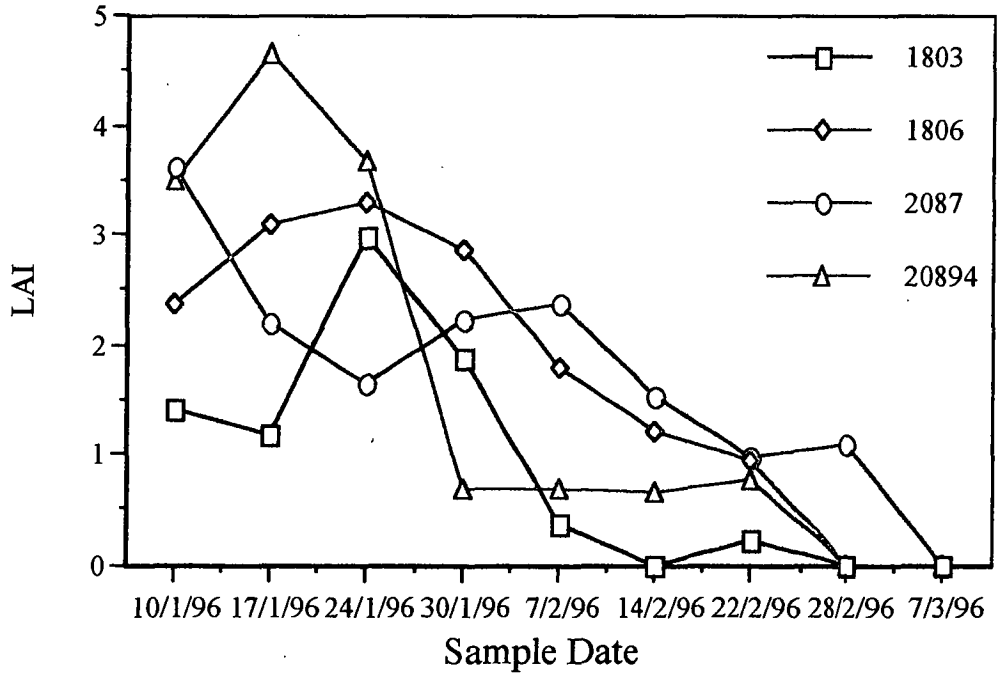


Figure 3.2. Leaf Area Indices for trial 2.



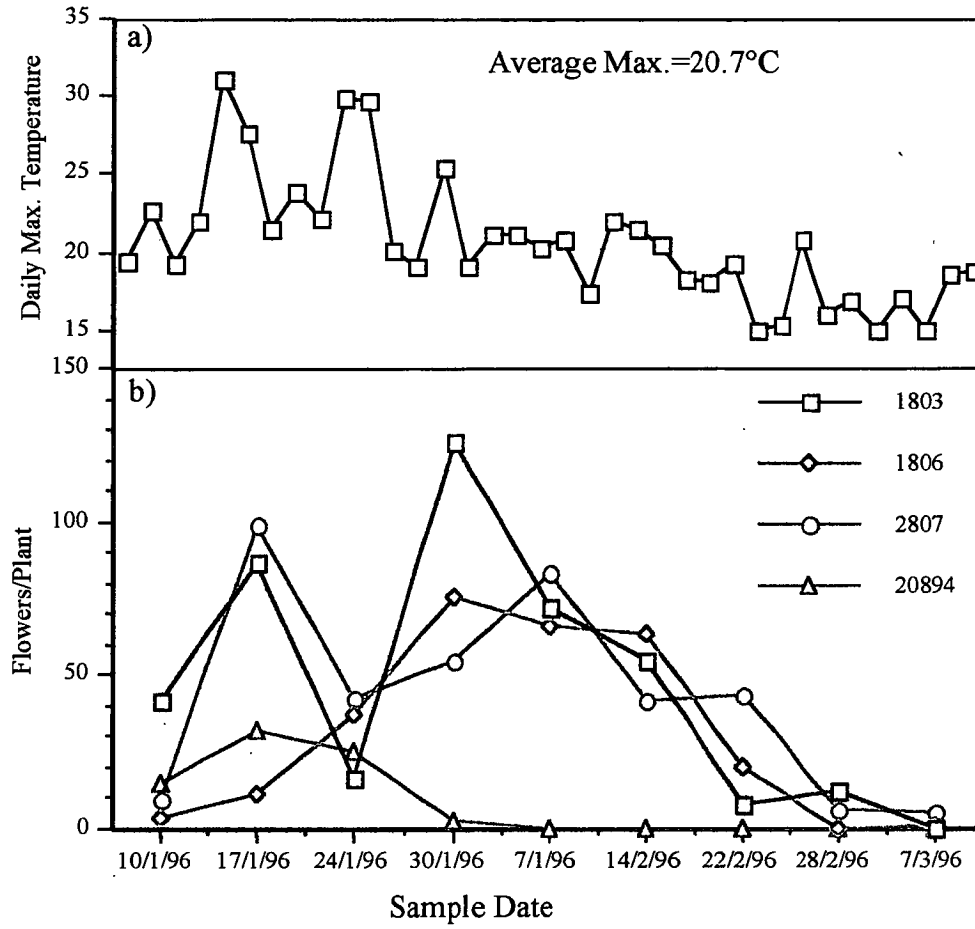
While the apetalous lines did not reach the same maximum LAI as 20894, they were able to maintain higher values of LAI throughout the flowering period, despite producing many more flowers than the petalled line (figure 3.3). This can be attributed initially to increased light penetration to the lower leaf canopy because of the apetalous flowers, but later it was most probably due to the absence of a substantial pod canopy to intercept radiation.

Figure 3.3 compares flower production of each of the lines on a flowers/plant basis with daily maximum temperatures over the flowering period. The apetalous lines 1803 and 2807 unexpectedly had in effect two peak flowering periods, the first of which coincided with days which reached temperatures of greater than 30°C. It is also obvious that the flowering period of all the apetalous lines was significantly longer than that of 20894.

Line 1806 had a more distinct flowering period than the other apetalous lines, though not as short as 20894. This may have been the result of this line avoiding the initial high temperature days experienced by 1803 and 2807, due its later flowering period.

Despite the large number of late flowers produced by the apetalous lines (figure 3.3), the high number of aborted pods produced by the apetalous lines suggests that these flowers were largely unproductive.

Figure 3.3. (a) Daily maximum temperatures experienced during flowering of trial 2 and (b) flowering periods, expressed on a flowers/plant basis.



Trials 1 and 3

Despite the problems encountered in trials 1 and 3, the results indicated that the conditions experienced by trials 1 and 3 during flowering and seed development were more conducive to higher apetalous yields than in trial 2. However, the yields produced by line 20894 were very consistent over the three sowing dates (table 3.3). Line 20894 produced more productive pods in trials 1 and 3 than in trial 2 resulting in slightly less seeds/pod being retained in those trials, but not to the extent of causing any yield reductions. During the flowering period of trial 1 (figure 3.4),

temperatures were lower than in trials 2 and 3 and there was only one day when temperatures approached 30°C. During flowering in trial 3 (figure 3.5) the average temperature was similar to trial 2, however, while there were several days which approached 30°C it was not exceeded. These high temperature days were separated by several days of lower temperatures.

In trials 1 and 3, line 1806 produced more seeds/pod than in trial 2 which, combined with higher productive pod numbers resulted in much higher yields. The trial 3 yield of 2243 kg/ha is the highest yield yet recorded for an apetalous *Ogura* A line. In both apetalous lines the number of aborted pods was much lower than in trial 2 which also suggests that conditions for pollination were more favourable in the early and late sowings.

It was clear over all sowing dates that the low number of seeds/pod was responsible for the low yields of the apetalous lines and that different sowing dates had a larger effect on these lines than on the petalled control.

Figure 3.4. (a) Daily maximum temperatures experienced during flowering of trial 1 and (b) flowering periods, expressed on a flowers/plant basis.

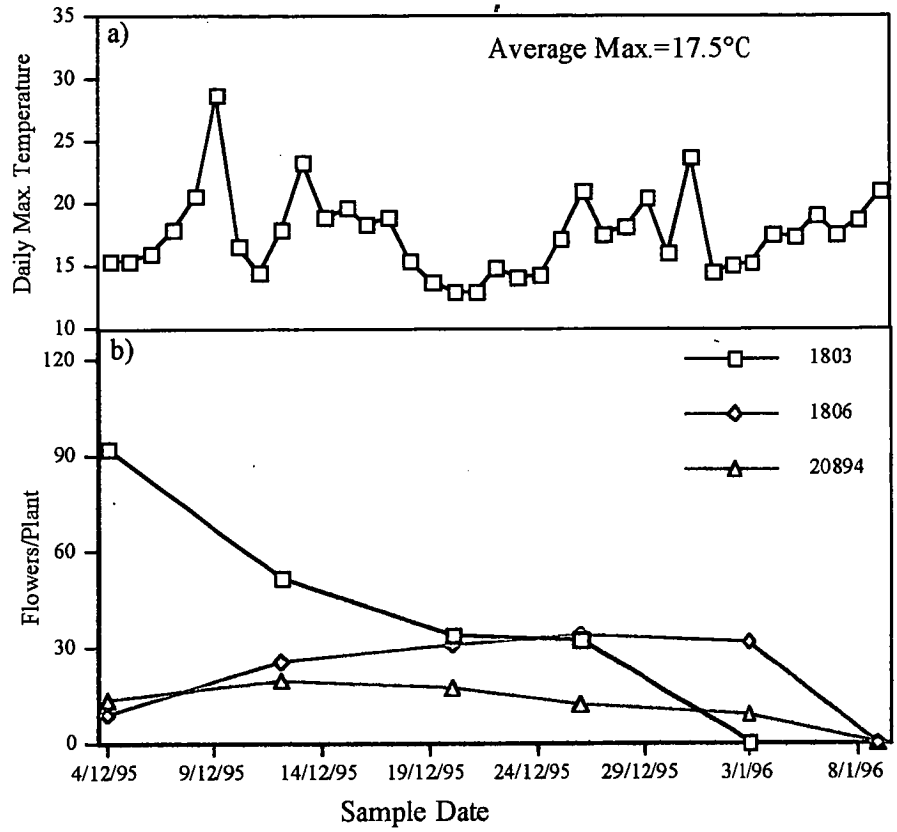
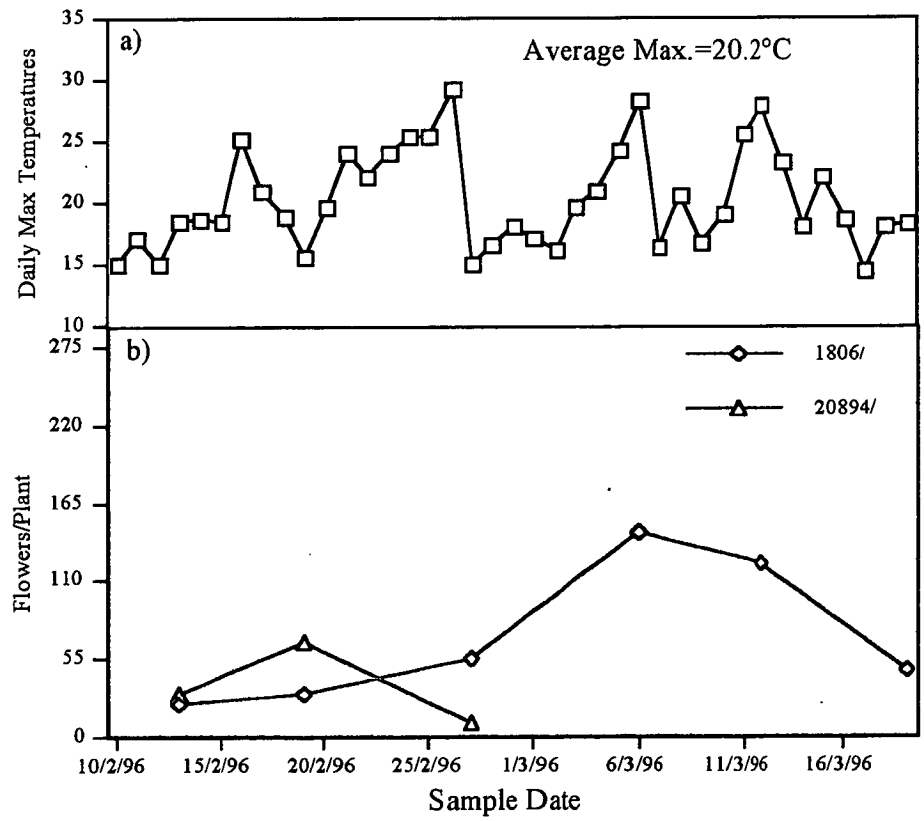


Figure 3.5. (a) Daily maximum temperatures experienced during flowering of trial 3 and (b) flowering periods, expressed on a flowers/plant basis.



Yield Components

Trial 2

As well as the overall yield components presented in table 3.3, components were separated according to tertiary, secondary and primary (including mainstem) branches (table 3.4). Data for some components of line 2807 were not available due to damage caused by mice to stored material.

The number of tertiary branches produced by lines 1803 and 2807 were markedly higher than for 1806 and 20894. The increase in branching appeared to be associated with the low number of productive pods produced on the primary and secondary branches by line 1803 and presumably 2807 (data not available). Although 1806 also had a similarly low number of productive pods it did not produce as many secondary and tertiary branches as the other apetalous lines. This appeared to be due to the shorter reproductive growth phase of 1806, which flowered two weeks later than the other apetalous lines but finished flowering at the same time (figure 3.3).

While 20894 produced most of its productive pods on primary and secondary branches, the apetalous lines produced most of their productive pods on tertiary branches. The percentage of flowers which went on to form productive pods in line 20894 showed a steady decline from 59% on the primary branches to 47% on the tertiary branches. However for the apetalous lines the percentage of productive pods produced from flowers was relatively constant over all branches, and considerably lower than line 20894 at all branching levels.

Table 3.4. Branch numbers and yield components expressed on a branch basis. Percentage of productive pods produced from potential pods in brackets.

Branches/Plant			
Line	*Primary*	*Secondary*	*Tertiary*
1803	5.6	14.3	18.6
1806	6.2	9.7	10.2
2807	6.0	15.9	22.9
20894	6.6	13.9	9.2
lsd(0.05)	*0.87*	*2.54*	*4.5*
Productive Pods/Branch Order			
1803	21.0(18%)	32.016%)	59.3(18%)
1806	17.1(16%)	28.8(22%)	42.9(22%)
20894	50.1(59%)	59.7(52%)	30.9(47%)
lsd(0.05)	*10.8*	*15.0*	*23.4*
Aborted Pods/Branch Order			
1803	97.2	162.1	273.8
1806	93.2	100.3	152.2
20894	34.5	55.3	34.6
lsd(0.05)	*15.3*	*32.3*	*80.8*
Seeds/Pod			
1803	3.0	3.4	2.9
1806	5.1	5.0	5.1
20894	19.7	21.8	18.3
lsd(0.05)=2.4			
1000 Seed Wt. gms			
1803	6.42	6.08	6.07
1806	6.17	5.60	5.09
20894	4.60	3.75	3.77
lsd(0.05)=0.21			

Flower production was clearly not a limiting factor for the apetalous lines as they had significantly more aborted pods at all branching levels in comparison with line 20894. Line 1803 was able to produce more potential pods than 1806 due a greater number of tertiary branches, however the number of productive pods set by line

1803 on tertiary branches was not significantly higher. This was reflected in the low percentage of productive pods set from potential pods.

In contrast to the apetalous lines which produced progressively more flowers on lower order branches, as indicated by the number of aborted pods, line 20894 had a similar number of aborted pods on the tertiary branches as in the primary branches.

The poor productivity of the apetalous lines was also apparent in the number of seeds produced per pod. As previously discussed the apetalous lines produced very few seeds/pod in comparison with line 20894. The number of seeds/pod produced on different branches remained very constant within lines, which suggests that assimilate supply was not limiting to the extent to cause seed abortion in the lower order branches.

A further indication that assimilate supply was not a limiting factor for the apetalous lines was the high individual seed weight produced by lines 1803 and 1806. All lines produced progressively lighter seed moving from the primary to the secondary and tertiary branches. The difference in seed weight probably reflected the shorter period for seed growth of the secondary and tertiary branches between flowering and maturity in comparison with the primary branches.

Discussion

The results from these trials illustrate how the individual yield components of the petalled and apetalous lines contributed to final yield. Clearly there were significant differences between yield components of the petalled and apetalous lines, which ultimately resulted in large yield variations.

The major limiting yield component of the apetalous lines was the low number of seeds produced per pod. In response to this the apetalous lines compensated by producing more flowers on a greater number of secondary and tertiary branches. It was clear from a visual inspection of the trials that the apetalous lines had a very high number of aborted or unproductive pods, so the rate of productive pod set would have been low as well as the rate of seed survival in those pods which did remain on the plant.

Seed yield and yield component data were not collected from B line plants used as the pollen source in this trial due to lack of resources and practical difficulties. The B line rows were situated in the middle of A line plots, and had a similar flowering period as the petalled A line (approximately three weeks). Therefore, by the time the apetalous A lines had reached maturity, up to four weeks later than the B line in some instances, most of the B line pods had shattered pods and lost seed. From visual observations, it was clear that apetalous B lines set pods and seeds normally, and had many fewer aborted pods than the respective A lines, and appeared to have a similar level of pod abortion as the petalled A line 20894. In hindsight a comparison of apetalous A and B line yield components would have been able to directly determine if the low number of seeds/pod and high number of aborted pods produced by the apetalous A lines was a result of female fertility or flower pollination problems.

The evidence again strongly suggested that vegetative growth did not restrict yields of the apetalous A lines, as growth rates were similar for all lines up to the beginning of seed fill. The apetalous lines showed greater leaf retention rates during the flowering period in comparison to 20894, which can be attributed to increased light penetration through the apetalous flower canopy. However, this was of little

benefit to the apetalous lines, having only a low seed load into which extra assimilate could be directed.

Compensatory growth of the apetalous A lines had the effect of increasing the flowering period. Line 20894 had a distinct flowering period lasting for about three weeks (figure 3.3 and 3.5), as did the apetalous B lines which are genetically identical to the A lines, except for the male sterile cytoplasm. However, flowering in the apetalous A lines was extended to five to seven weeks with differences between the early and late flowering lines. The petalled line 20894 had a more extended flowering period in trial 1 (figure 3.4) which may have been due to the milder weather conditions experienced during flowering.

The compensatory growth exhibited by the apetalous lines appeared to be the result of reduced sink capacity caused by poor seed set. The response was similar to the results previously described by Tommey and Evans (1992), however, in the case of the apetalous lines the reduction in sink capacity occurred over the whole plant and was not restricted to specific branches.

Keiller and Morgan (1988) suggested cessation of flowering occurred once demand from pods for assimilates became greater than that from the apices of the branches. Reduced sink capacity in the apetalous lines resulted in more assimilates becoming available to the secondary and tertiary branches. This resulted in significantly more branching of the apetalous lines and consequently continued flowering on these branches after line 20894 had finished. However, the extra potential pods produced were unable to compensate for the low number of seeds set by the main stem and primary branches. This agrees with the results of Tommey and Evans (1992), which demonstrated that plants, which had flowers removed from the main stem and

primary branches, were unable to compensate by the production of more potential pods on lower order branches.

The analysis of yield components on a branch order basis (table 3.4), highlighted significant contrasts between the apetalous and petalled line, which appear to be more a function of the apetalous character than of genetic differences. The apetalous lines had very similar yield components, despite having quite distinct genetic backgrounds (apart from the common ancestor RU6/RU9 cross), which suggests a strong apetalous character influence. The percentage of productive pods set from potential pod sites remained constant over all branch orders in the apetalous lines. Line 20894 displayed an expected pattern with a gradual reduction in the percentage of pods set moving from primary to tertiary branches. This can be explained by the greater sink strength exerted by pods on primary branches, and is also reflected in individual seed weights.

The fact that the apetalous lines did not show a similar pattern indicated that some other factor is influencing yield components, other than the typical assimilate source/sink relationship evident in the petalled line.

The petalled A line obviously did not suffer the same problem of low pod and seed set, although it too was totally reliant on cross-pollination by an insect vector. As this line also contains the *Ogura* cytoplasm, the CMS system itself cannot be attributed to low pod and seed set. As previously mentioned, although data were not collected from apetalous B line plants in this trial, they clearly did not suffer the same low level of pod and seed set as the apetalous A lines.

Taking these factors into consideration, it would appear that the main reason for the high number of aborted pods and low number of seeds/pod produced by the

apetalous A lines was related to pollination of the apetalous, male-sterile flower. However, with the data available from trials thus far, low female fertility, defined as the number of fertile ovules produced per pod, as a cause of low seed set can not be eliminated at this stage.

There are several other factors that also appeared to be influencing the yields of apetalous A lines.

Due to the extended flowering period of the apetalous A lines, pollen availability must also be taken into consideration. Self-pollination of the apetalous B lines ensured good pod set and a contracted flowering period, with the flowering period finishing at around the same time as line 20894. Therefore, for much of the extended flowering period of the apetalous A line plants, it would be expected that only low levels of pollen were available for pollination. The design of the experiments was such that some degree of cross-pollination would have undoubtedly occurred. However, it would appear that external pollen sources had very little influence on final yields. The lines used in this trial all had the same flowering period, with the exception of line 1806. Line 1806 reached peak flower at a stage when other B lines had finished flowering, and so would have received the least amount of pollen from other sources. Despite this, the A line of 1806 produced the highest yields of the apetalous lines in all the trials conducted in this season, suggesting that the effect of cross-pollination between different lines was negligible.

Results from trials 1 and 3 indicated that the apetalous A lines are influenced to a greater extent by environmental conditions than the petalled line 20894. The higher temperatures experienced during trial 2 may have been responsible in part for the high number of aborted pods produced by the apetalous lines. In lines 1803 and 2807 the occurrence of hot days appeared to cause a temporary cessation and then

extended the flowering period as the plants attempted to compensate for low seed set.

As previously discussed, heat stress during flowering of summer rape has been shown to reduce both female and male fertility. The temperatures reported by Morrison (1993) which caused reductions in seed set were exceeded on several days in trial 2. The reason that the petalled line 20894 was not affected by the high temperatures may be that the reflection of sunlight by petals assists in keeping the average flower temperature lower than in the apetalous flowers. While flower canopy temperature was not investigated in these trials, it could be measured easily and accurately with an infrared thermometer to determine if there are in fact significant differences between the petalled and apetalous flower canopies.

Chapter 4.

The Effect of Hand Pollination and Bee Behaviour

Objectives

The trials conducted during this season of trials (1996/97), had a similar design as in previous years: the same lines were used, plus an additional apetalous line, 2809 that had shown some promise in the 1994/95 trials but was unavailable in 1995/96. The emphasis was on determining the reason for the poor seed set of the apetalous A lines and the high incidence of pod abortion, these being the components identified as limiting to yield. The similar manner in which final yield was produced across the apetalous A lines despite their having varied genetic backgrounds, suggested that there were common factors which were affecting the number of seeds/pod and pod abortion. The apetalous character could have caused poor pollination of A line flowers, or the presence of the *Ogura* CMS may have resulted in a negative influence on female fertility.

Introduction

Seed production of an angiosperm flower depends initially on a quantity of viable pollen reaching its stigmatic surface (Waser and Price, 1991). Subsequently a pollen grain must produce a pollen tube that grows to the ovary and fertilises an ovule with the resulting embryo successfully developing and maturing. For oilseed rape the potential number of ovules/pod may vary between 18 and 35 depending on cultivar (Mendham *et al.*, 1984), with each ovule requiring a successful pollen grain for fertilisation. Therefore the final number of seeds/pod in canola depends on the

number of ovules/ovary, the number of ovules fertilised and the number of fertilised ovules which develop into seeds (Pechan, 1988).

The number of ovules is always greater than the number of seeds at harvest, which indicates that this is not a limiting component. This suggests that fertilisation or subsequent abortion limit seed numbers. There appears to be two main causes for seed abortion. Competition for assimilate supply as described by Mendham *et al.* (1981), affects seeds which have begun development and occurs in crops with heavy seed loads. This competition leads to ovules with the highest assimilate sink strength progressing to maturity at the expense of others. The other stage at which ovule abortion may occur is during flower development, when ovules fail to develop and degenerate shortly after flower opening (Bouttier and Morgan, 1992).

In situations where the amount of pollen is not limiting, Bouttier and Morgan (1992) showed that the number of seeds/pod appeared to be determined by the number of ovules which contained fully developed embryo sacs at flower opening. In their study 64% of ovules contained a complete and normal embryo sac. The most critical stage for ovule fertility appeared to be when the buds were 3-4 mm long, about 7-8 days before flower opening. This corresponded with meiosis II and/or the early differentiation of the uninucleate megaspore (Bouttier and Morgan, 1992). The successful development of a complete and normal embryo sac was not linked to any external influences, but appeared to be determined by the genetic influence on the plants in question.

Previously it was reported (Pechan, 1988) that abortion of ovules post pollination in flowers with adequate pollination was due to the failure of pollen tubes to penetrate and fertilise the ovule. However the high level of agreement between the number of ovules with a fully developed embryo sac and the number of seeds at harvest shown by Bouttier and Morgan (1992) would suggest that the successful development of

the embryo sac is generally the most important factor under normal conditions where fertilisation is not limiting.

In hybrid seed production the amount of pollen deposited on the stigma of A line male sterile flowers is also a factor limiting seed set as well as those factors just discussed. This is further complicated in the apetalous A lines for which the floral morphology has been radically altered. It is highly probable that the removal of petals affects the behaviour of honey bees which are the usual vectors for pollination.

While Morrison (1993) showed that pollen viability may reduce seed set in plants grown at high temperature, pollen sterility was dismissed as a cause for low seed set in non-stressed plants by Pechan (1983) and Ancha (1988) cited in Bouttier and Morgan (1992).

Changing flower morphology in a manner less drastic than the removal of petals has been reported to modify bee behaviour by increasing the incidence of honeybees 'sideworking' flowers. This may result in flowers not being pollinated. Honey bees observed foraging on the oilseed rape variety 'Nilla' by Free and Ferguson (1983) were all reported to enter the top of the flower and become dusted with pollen as contact was made with anthers and the stigma. However 20% of honeybees foraging on the variety 'Primor' were found to 'sidework' flowers by inserting their tongues between the base of the petals and sepals to obtain nectar. When a flower is visited in this manner the chances of pollen being picked up from anthers or deposited on the stigma are greatly reduced. This change in honeybee behaviour was believed by Free and Ferguson (1983) to be a result of the different flower size and structure of 'Primor'. Free and Williams (1973) reported that Brussels sprout (*Brassica oleracea* L.) varieties with large flower parts were more likely to be sideworked as honey bees were unable to reach the nectaries from the top of the flower. The incidence of

sideworking increased as flowers became older and the floral organs spread apart, allowing the nectaries to be accessed from the side of the flower.

Observations made on apetalous and petalled rapeseed flowers by Pierre (1995) indicated that in 50% of visits made to apetalous flowers, honeybees failed to enter the flower, and obtained nectar by sideworking. On petalled flowers by comparison, in 80% of visits bees would move over the stigma and anthers to visit nectaries. It was also reported in this study that there were no significant differences in the number of honeybees visiting the apetalous and petalled line. However honeybees returned consistently to one flower type and there was very little movement between the two lines.

While the sideworking of flowers by honeybees has little effect on the yield of male fertile plants which are able to self-pollinate, it may significantly affect seed production on A line plants which depend on insect vectors for pollination. It was suggested by McVetty *et al.* (1989) that the smaller anthers and filaments, and greatly reduced petals of *polima* A line flowers would increase the likelihood of leaf cutter bees (*Megachile rotunda*) sideworking these flowers. The altered morphology of the *polima* A line flowers resulted in the nectaries being readily accessible from the side of the flower. There were large differences between A lines in the percentage of flowers sideworked: in two cultivars 60% of flowers were sideworked while in the other two cultivars this occurred in less than 10% of flowers visited. In the respective B lines where flower morphology was considered normal, less than 10% of flowers were sideworked.

The yields of A lines in which more flowers were sideworked were not affected by this bee behaviour. This was attributed to high bee numbers that ensured numerous visits to each flower which increased the chance of successful pollination. No

suggestion was given to explain why a higher number of flowers were sideworked in two of the A lines investigated.

Methods

The trials were sown with an eight row precision cone seeder which was calibrated for a target density of 100 plants/m^2, with rows spaced at 0.15 metres. Each plot consisted of sixteen rows of A line (comprising a subplot), planted on either side of four rows of B line constituting 36 rows in total.

Each plot was fifteen metres long and randomly replicated within two blocks. Within blocks plots were sown linearly and blocks were positioned side by side with rows running parallel in respective blocks. In the 0.5 metre area between the B and A line rows, two rows of barley were planted to prevent weeds from becoming established. In the three-metre area between plots and blocks, A line plants were used as 'fillers' to reduce pollen transfer between lines.

The same level of nutrients was applied as in the 1995/96 trials and agronomic details are presented in table 4.1. An adjacent paddock growing seed carrots contained five hives of honeybees, which were located 50 metres from the canola plots, this provided the main source of pollinators.

Loktronic™ tensiometers were placed in blocks to assist in irrigation management so plants did not experience any periods of severe water stress. Plots were top dressed by hand with nitrogen at the beginning of stem elongation (table 4.1).

For the final harvest the outer row of each plot situated the furthest from the B line rows was discarded and a five metre section from the middle of each remaining row was cut by hand, labelled and bundled together for drying. Bundles were threshed

individually with a mechanical plot thresher allowing yield and 1000 seed weight to be calculated on a row basis. This data will be discussed in chapter 6. Total yield was calculated from the summation of fifteen individual row yields, constituting 11.25 m^2 per subplot in total. The number of productive pods, aborted pods and seeds/pod was calculated from five randomly sampled plants.

Table 4.1. Agronomic procedures for trials conducted in 1996/97 at the University Farm, Cambridge, Tasmania. (a.i. = active ingredient)

Sowing date	15/10/96
Fertiliser	
Pre-drilled (9/10/96)	N : 49.5 kg/ha P : 40.0 kg/ha K : 75.0 kg/ha S : 39.0 kg/ha
Top dressing	N : 90.0 kg/ha (29/11/96)
Weed Control	
Trifluralin	600 g a.i. /ha (9/10/96)
Clopyralid	75 g a.i. /ha (26/11/96)
Pest control	
Red Legged Earth Mites (*Halotydeus destructor*)	Omethoate 14.5 g a.i. /ha (20/10/96)
Cabbage Moth/Heliothis (*Plutella xylostella*) (Family Noctuidae)	Permethrin 75 g a.i. /ha (8/11/96) Chlorpyrifos 600 g a.i. /ha (17/1/96)
Slugs	Methiocarb 110 g a.i. /ha (29/10/96)
Final Harvest	Line 1803 (17/2/97) 1806 (7/3/97) 2807 (26/2/97) 2809 (27/2/97) 20894 (14/2/97)

Hand Pollination Experiment

This experiment was conducted to determine if pollination was a limiting factor for A line seed production.

Ten plants from each line were numbered with a plastic tag on the mainstem, positioned under the most recently opened flower. The plants selected had twenty to forty unopened flower buds above the tagged flower on the main stem (MS).

Every second day flowers above the tag on the MS were hand pollinated by taking a flower from the respective B line and rubbing an anther on the stigma of each flower, until it was possible to see pollen on the stigmatic surface. If there were any flowers in which the style was caught in the tips of the sepals, it was carefully released with a dissecting needle and then hand pollinated.

One week after flowering on the MS had finished, when the youngest pods that had been hand pollinated were over two centimetres long, five of the tagged plants from each line were harvested. Five plants, which had not been hand pollinated, were matched for age with the tagged plants and harvested as the control treatment. The number of pods containing seeds and the number of aborted pods on the MS of the tagged plants after hand pollination commenced were counted. These components were also determined on the control plants on a section of MS with a similar number of potential pod sites.

Five pods from each plant were removed from the hand pollinated section of the MS, and corresponding part of the MS of the control plants. The pods were dissected to determine the number of fertile and infertile ovules contained in each.

At final harvest the remaining tagged plants were harvested, again with five control plants, and the number of productive pods, aborted pods and seeds/pod were counted.

Bee Observations

In order to confirm that the apetalous flowers attracted a similar number of bees as did the petalled line, the number of bees observed working on each line were counted. This was achieved by marking out a one metre square area in each plot, then walking up and down the plots and counting the number of bees within the marked area while walking past over three observation periods.

The manner in which bees interacted with A line flowers was also investigated. Bees were observed as they visited flowers, and a flower visit was recorded as successful if at any stage during the visit the bee made contact with the stigma (W. Balch, Pacific Seeds Toowoomba, Qld., pers. comm.).

Results

Total Yield and Yield Components

Plot yields were obtained from plants pollinated under natural conditions and did not include hand pollinated plants.

As in previous trials the apetalous lines produced significantly lower yields than the petalled line 20894 (table 4.2). Line 20894 produced a high yield for a male sterile A line of 3644 kg/ha, more than twice that of the highest yielding apetalous line

1803. There were also significant differences in yields produced by the apetalous lines ranging from 1773 kg/ha for line 1803 to 529 kg/ha for line 2807.

The plants selected for the determination of pods/m^2 gave a much higher estimate of this yield component than what was acceptable based on the yields obtained. This appeared to be due to those plants selected for hand pollination and control plants producing more productive pods than the general population. Consequently the numbers of productive and aborted pods have been expressed on a per plant basis to describe trends which help to explain yield differences.

Table 4.2. Yield and yield components for 1996/97 trials. The percentage of productive pods produced from potential pods are presented in brackets. (NS=No Significant Differences).

			Pods/plant		
	Yield kg/ha	*1000 Seed Wt.g*	*Productive*	*Aborted*	*Seeds/pod*
1803	1773.8	5.05	280(52%)	254	10.2
1806	1395.9	5.22	226(48%)	241	7.5
2807	529.9	4.64	148(20%)	581	6.7
2809	1288.1	5.49	191(33%)	388	6.3
20894	3644.5	3.75	165(83%)	34	21.9
lsd (0.05)	*255.0*	*0.36*	*NS*	*203*	*2.8*

The yield components (table 4.2) confirmed the results of previous trials which indicated that the poor performance of the apetalous lines was due to a much lower number of seeds/pod in comparison with the petalled line 20894. While the data on productive pods are not conclusive they do suggest that the low yield of line 2807 was due to it setting fewer productive pods than other lines, as well as having lighter seed than most other lines. In comparison, line 1803 produced more productive pods than other lines and produced more seeds/pod than the other apetalous lines, both factors contributing to its high yield among the apetalous lines.

The petalled line 20894 again showed how high yields can be obtained through efficient reproductive growth. This line produced a relatively low number of productive pods/plant, retained a high number of seeds/pod and had very few unproductive pods. Due to the large seed load individual seed weight was quite low in comparison to the apetalous lines, but this was more than compensated for by the high number of seeds produced.

Hand Pollination Experiment

Pod Production

Table 4.3 contains the data on the percentage of productive pods set from potential pod sites on the mainstem of hand pollinated and control plants. It was possible for pollination to occur on the control plants by wind or insect means. Before analysis of variance the data were subjected to an arcsine transformation, however, percentages have been used for presentation. Presentation of data as the percentage of productive pods set eliminated the difficulty of comparing mainstems which may have had a different number of flowers. Sample A was taken shortly after flowering while sample B was taken at final harvest (as explained in the Methods section).

All of the apetalous lines showed an increase in the number of pods set after hand pollination at both sample times (figure 4.1), but not all differences were statistically significant. In contrast the petalled line 20894, showed no response to hand pollination at either sample time, nor in the combined analysis of samples A and B.

All of the apetalous lines showed significant differences between the pollinated and control treatments when the data from both sample times were combined. In addition when the data of all lines were combined and analysed according to sample

time, there were significant differences apparent between treatments even with the inclusion of the petalled line, 20894. This suggests that the sample size was not large enough to show significant differences between hand pollination and control treatments in some of the apetalous lines despite large differences in the percentage of pods produced.

The results do indicate that lines 2807 and 2809 did respond more strongly to hand pollination than the other apetalous lines, as both lines showed significant differences for sample A, despite the small sample size.

While not conclusive, the data indicates that the majority of pods set at flowering in both the hand pollinated and control treatments were carried through to maturity in all the lines investigated in this trial. This suggested that the reason for the high number of aborted pods produced by the apetalous lines in the control treatment is the result of lack of pollination, and was not caused by pod abortion post flowering.

Table 4.3. The percentage of productive pods set from potential pod sites on the main stem of hand pollinated and control plants. Significant differences between lines within treatments are denoted by superscripts, where means with the same superscript are not significantly different. Underscoring indicates means that are not significantly different for treatments and sampling times within lines and line means.

	Sample A		Sample B		Sample A+B	
	Pollinated	*Control*	*Pollinated*	*Control*	*Pollinated*	*Control*
1803	63.5^{b}	48.3^{bc}	76.3^{b}	63.4^{b}	70.1^{b}	55.9^{bc}
1806	81.9^{a}	66.8^{ab}	78.0^{bc}	56.5^{b}	80.0^{a}	61.7^{b}
2807	76.4^{a}	47.9^{c}	74.0^{bc}	46.9^{b}	75.2^{a}	47.4^{c}
2809	67.5^{a}	39.8^{c}	59.0^{c}	46.7^{b}	63.2^{b}	43.2^{c}
20894	73.8^{a}	79.0^{a}	91.5^{a}	81.4^{a}	83.6^{a}	80.2^{a}
Means	72.6	56.3	75.7	60.00	74.4	57.7
Combined Means	64.5		67.4			

(Transformed data and LSDs are presented in appendix C)

Figure 4.1

Main stems of line 2807. Flowers above the orange tag were hand pollinated, the mainstem on the right was a control plant.

Ovule Production

The total number of ovules/pod produced by each line was calculated by combining the number of fertile and infertile ovules of the pods examined in sample A (table 4.4). There were no significant differences between treatments for any of the lines, indicating that the technique was able to detect the majority of ovules present in both treatments. Generally all lines produced a similar number of ovules, with the exception of 1803, which produced fewer ovules/pod that all other lines. Despite this line 1803 set more seeds/pod than any other apetalous line (table 4.2).

Table 4.4. Total number of ovules/pod estimated by counting fertile and infertile ovules contained in pods at sample A.

	Sample A	
	Pollinated	*Control*
1803	25.6	22.7
1806	31.6	33.3
2807	30.4	30.0
2809	29.1	25.6
20894	32.6	30.0
	lsd(0.05)=4.1	
Means	*29.9*	*28.3*
	lsd(0.05)=1.7	

Hand pollination clearly increased the number of fertile ovules/pod in the apetalous lines at the stage when sample A was taken (table 4.5 and figure 4.2), while it did not appear to have an effect on the petalled line. After hand pollination the number of fertile ovules/pod produced by the apetalous lines, were nearly as great as in the petalled line 20894, with only 2809 having significantly less. In contrast under natural pollination conditions (control treatment), all apetalous lines produced significantly fewer fertile ovules/pod than 20894.

The response to hand pollination as seen in the A samples, differed between the apetalous lines. The difference between treatments for line 1803 was not significant, mainly due to the ability of this line to fertilise a moderate number of ovules in the control treatment. Among the apetalous lines 1803 also had the highest number of seeds/pod at harvest in the overall plot results, which were calculated from pods over the whole plant.

The effect of hand pollination on lines 2807 and 2809 in increasing the number of fertile ovules/pod was much greater, mainly because the control treatment of these lines had very few fertile ovules/pod. However, even with hand pollination line 2809 had less than 50% of its ovules fertilised.

Figure 4.2. A pod produced from a hand pollinated flower (top) compared with a pod which was pollinated naturally (control), both pods from line 1803.

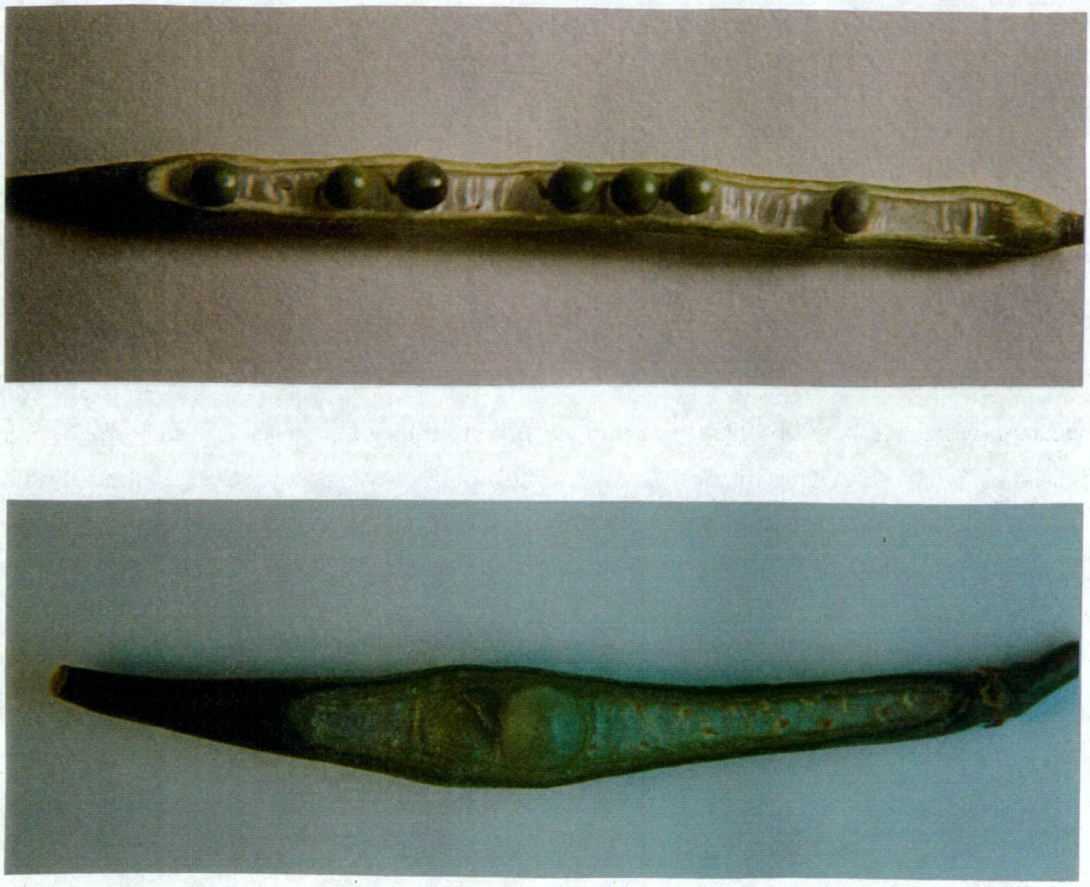


Table 4.5. The number of fertile ovules/pod at sample A and the number of seeds/pod at harvest (sample B). Results were obtained from pods sampled from the mainstem.

	Sample A		*Seeds/Pod* Sample B		Sample A+B	
	Fertile Ovules/Pod					
	Pollinated	*Control*	*Pollinated*	*Control*	*Pollinated*	*Control*
1803	17.8	12.6	14.9	14.8	16.4	13.7
1806	18.8	12.9	10.9	5.4	14.8	9.2
2807	19.2	6.5	21.2	6.7	20.2	6.6
2809	14.7	6.8	8.8	7.5	11.7	7.2
20894	23.6	21.0	22.8	21.8	23.1	21.3
		lsd(0.05)=5.8			*lsd(0.05)=4.1*	
Means	*18.8*	*12.0*	*15.7*	*11.24*	*17.3*	*11.6*
		lsd(0.05)=2.0			*lsd(0.05)=1.8*	
Combined Means	*15.4*		*13.5*			

There was considerable variation between lines in the number of seeds retained to harvest (sample B), with 2807 being the only line to maintain a significant difference between treatments. Line 2807 maintained the same number of seeds/pod it had in both treatments at sample A through to harvest, as did the petalled line 20894. The remaining apetalous lines retained fewer seeds to harvest in one or both treatments.

Line 1806 lost more than 20% of fertile ovules in the time between sample A and B in both hand pollinated and control plants. This line had fewer seeds/pod in sample B than the overall plot yield component value (table 4.2), while the other lines had similar or slightly higher numbers. Considering this, the low number of seeds retained in both the control and hand pollinated treatments was most likely to have been due to experimental error as a result of the small numbers of plants sampled.

Line 2809 also lost a significant number of fertile ovules/pod between the two sampling periods in the hand pollinated plants, so that at harvest the number of seeds/pod for the two treatments was very similar.

The results just described indicate that there was considerable variation in the ability of the apetalous lines to retain seeds through to final harvest. While vegetative growth data were not collected for this trial, previous results and visual assessment indicated that differences in general plant vigour did not appear to be responsible for low rates of seed retention. Therefore, it appeared that some factor associated with female fertility, which was originally encountered with the production of *Ogura* lines, was having a significant influence.

This became more evident when the female fertility was calculated from the total number of ovules, determined at sample A, and the number of fertile ovules or seeds

counted at sample A or B from the hand pollinated treatment (table 4.6). This calculation was based on the assumption that the ovules which failed to develop in flowers which were hand pollinated did not contain a functional embryo sac as reported by Bouttier and Morgan (1992). For analysis of variance the percentage data were transformed, however the percentages are presented in table 4.6. Presumably by sample B the number of seeds/pod was not only dependant on female fertility, but also the abortion of seeds post-flowering.

There were no significant differences between lines in female fertility levels from the data collected at sample A. However, lines 1806 and 2809, which had the lowest levels of female fertility at sample A had significantly lower levels at final harvest (sample B) in comparison with the other lines. The losses that occurred in 1806 and 2809 after sample A would have been the result of seed abortion, in addition to ovule abortion that was observed in all lines. This did not occur in other lines, indicating a difference in seed retention ability. The fact that the other apetalous and petalled lines did not exhibit this characteristic though they possessed *Ogura* cytoplasm indicated that it was not a factor directly caused by the CMS trait, or the apetalous character.

Table 4.6. Female fertility calculated for samples A and B hand pollinated treatments. Significant differences are denoted by superscripts, where means with the same superscripts both within and between sample times are not significantly different. (Transformed data and LSDs presented in Appendix C).

	Percentage Female Fertility	
	Sample A	Sample B
1803	69.6[a]	58.2[a]
1806	59.4[a]	34.4[b]
2807	63.2[a]	69.6[a]
2809	50.6[a]	30.1[b]
20894	72.2[a]	69.8[a]
Means	*63.0*	*52.4*

Bee Observations

The results presented in table 4.7 indicate that there were no significant differences in the number of bees observed on the apetalous and petalled lines. However, the apetalous flower characteristic had a major influence on the manner in which bees interacted with flowers. Data on the proportion of successful bee visits made to flowers were subjected to an arcsine transformation so analysis of variance could be calculated, and the percentage of successful visits is displayed in table 4.7.

Table 4.7. The number of bees/m^2 recorded on the respective lines and the percentage of successful bee visits. Significant differences ($P<0.05$) are denoted by superscripts.

	1803	*1806*	*2807*	*2809*	*20894*
Bees/m^2	2.1	2.3	1.7	2.6	1.9
S.E.=0.4					
% of Successful Bee Visits	$35\%^{bc}$	$45\%^{b}$	$33\%^{bc}$	$15\%^{c}$	$85\%^{a}$

(Transformed data and LSDs presented in appendix C)

The presence of petals obviously promoted contact between bees and the stigma resulting in significantly more bee visits being recorded as successful on the petalled line 20894. The incidence of sideworking also varied between the apetalous lines (table 4.7). The highest proportion of successful visits (45%), was made on line 1806, while for line 2809 in only 15% of visits did the bee make contact with the stigma. This may have contributed to this line setting the lowest number of productive pods in the control treatment (table 4.3).

The flower petals of line 20894 in effect provided a landing platform for the bee (figure 4.4a), so that when it landed on top of a flower it made direct contact with the stigma in 85% of visits. In the case of an apetalous flower, the strong directional influence provided by petals to encourage the bee to make contact with the stigma

was absent. Instead, bees were more likely to land on the side of the flower (figure 4.4b) and move around the sepals to reach the nectaries, rather than climb over the top of the stigma.

The differences in the proportion of successful flower visits between the apetalous lines also appeared to be related to the length of the style and how far the sepals had opened. If the style protruded well beyond the tip of the sepals then the likelihood of the bee touching the stigma was reduced as during a visit the bee concentrated on the nectaries located at the base of the flower. There also appeared to be some variation between the lines in the degree that sepals opened. Sepals that opened wider prompted bees to land on top of the flower, which made contact with the stigma more frequent, provided the style was not too long.

a) b)

Figure 4.4. (a) Petals provide a landing platform for bees, (b) while on apetalous flowers bees are more likely to land on the side of the flower and not make contact with the stigma.

Discussion

Data on pod and seed set following hand pollination of apetalous flowers clearly showed that they not adequately pollinated under natural conditions, resulting in a lower percentage of productive pods being set from potential pod sites. In contrast

pollination did not appear to be a limiting factor for the petalled line 20894 as it showed no response to hand pollination.

The number of ovules fertilised per pod also increased in the apetalous lines after hand pollination indicating that this was also a limiting factor for seed production. However the ability to carry fertilised ovules through to maturity varied between the apetalous lines, while there was little variation in the percentage of productive pods retained to harvest. Figure 4.6a illustrates how the initial effect of hand pollination was to increase the number of ovules/pod and productive pods in the apetalous lines, but by final harvest (figure 4.6b) the main effect was only an increase in the number of productive pods. The exception was in line 2807, which retained the extra ovules produced from hand pollination.

The results indicated that the apetalous lines, with the exception of 2807, suffered some problems with seed abortion. That line 2807 did not appear to have this problem suggests that seed abortion was not a direct consequence of the apetalous character. A comparison of the apetalous B line's female fertility would have proven this conclusively, however the relevant data were not collected in these trials. The high ovule retention rate displayed by the petalled line 20894 also eliminated the possibility that the *Ogura* CMS was responsible.

There was some indication that the respective genetic backgrounds of the lines used in this trial significantly influenced seed retention ability. By the time of final harvest (sample B) both 1806 and 2809 had significantly lower levels of female fertility than the other lines (table 4.6). Both of these lines were developed from NSW lines (table 2.1) and thereby more closely related than other lines.

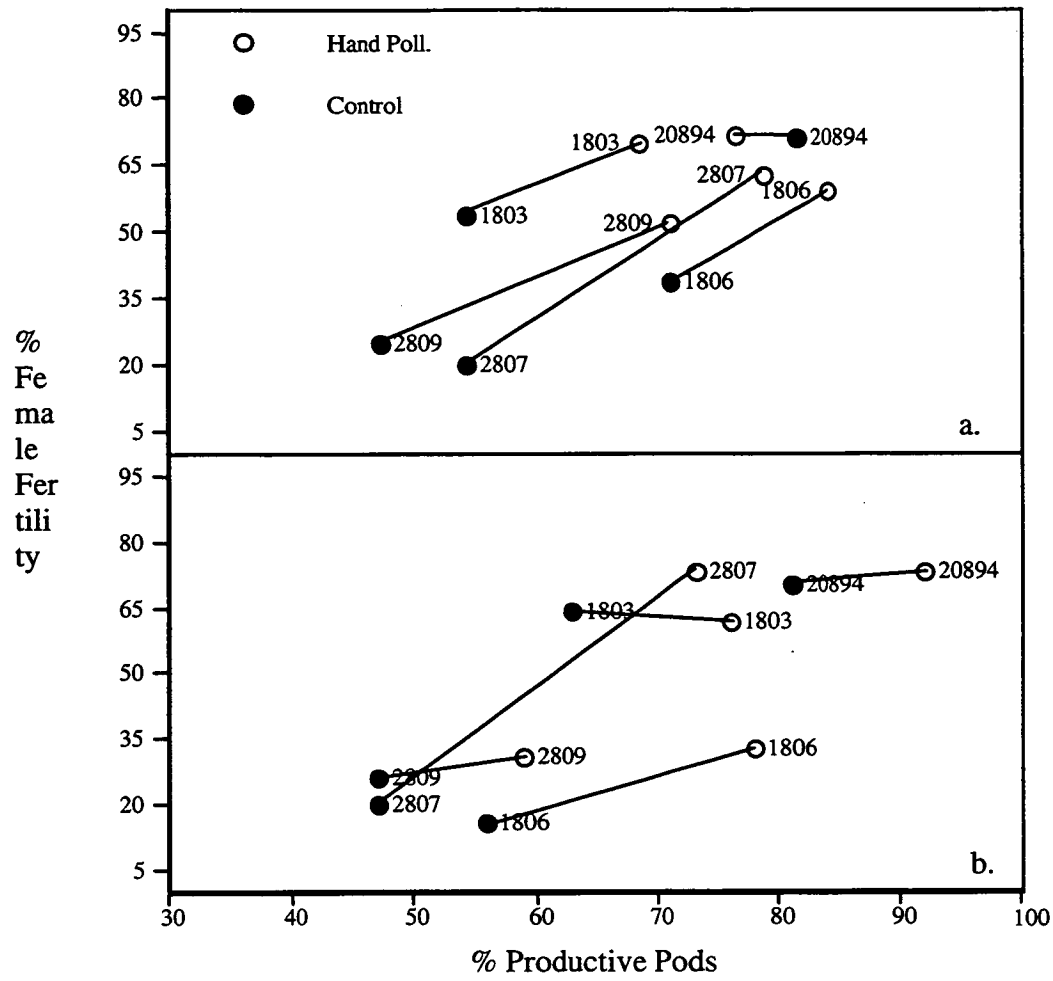


Figure 4.6. Female fertility (table 4.6) plotted against percentage of productive pods (table 4.3). (a) after flowering, (b) final harvest.

Normally seed abortion which occurs after the pollination stage is caused by intense competition for assimilate between developing seeds in crops which have set a heavy seed load. This seems unlikely, for a number of reasons, to be the cause of seed abortion between sample A and final harvest for line 1806 and 2809.

Both lines produced only modest yields, and it would be expected that increased radiation penetration into the crop canopy as a result of apetalous flowers would assist in seed retention, as reported by Rao *et al.* (1991). The pods that were sampled were also from the mainstem which should be a stronger sink for assimilate than pods on lower order branches (Keiller and Morgan, 1988). Evidence that pods

on the mainstem received a good supply of assimilate was also demonstrated in chapter 3 (table 3.4) where seeds from the mainstem and primary branches were significantly heavier than seeds produced on secondary and tertiary branches. The information gained from the previous trials also eliminates the possibility that seeds are deprived of assimilates due to extensive branching and flower production of the apetalous lines. If this occurred then it would be expected that line 2807 would have also suffered seed abortion, as it produced the most compensatory growth in terms of flower production. While line 2807 did produce the lightest seed of the apetalous lines, it did not have a reduction in seed number between sample A and final harvest.

From the observations of bee behaviour it appears that the high percentage of apetalous flowers sideworked by bees was responsible for the lack of pollen being deposited on the stigma. This would explain why the apetalous lines responded strongly to hand pollination to produce more pods and seeds/pod. McVetty *et al.* (1989) reported that yields were not reduced in *polima* A lines where sideworking by bees was more common. In the present study yield differences between A lines could not be attributed to differences in bee behaviour, however it would appear that it was a major factor governing the yield difference between the apetalous lines and the petalled line.

Although the effect of sideworking became obvious in the most extreme situation when apetalous and petalled flowers were compared, the differences between apetalous lines in the structure of flowers were much more subtle. Differences in bee behaviour, which were ultimately measured in yield, were not as distinct.

Despite the relatively low yields of the apetalous lines the results do indicate that better pollination would lead to yield improvements. A greater proportion of productive pods produced from potential pod sites and more seeds/pod would be

responsible for the effect. However, it needs to be determined why seed abortion occurred in some apetalous lines. As discussed in Chapter 1, low female fertility problems encountered in developing *Ogura* restorer lines was attributed to the retention of radish genetic material (Delourme and Eber, 1992). If a similar situation was occurring in the A lines investigated in these trials then it should be possible to improve female fertility with a breeding program. It is important to have lines that have good seed retention ability, as in order to obtain high yields it is vital that any ovules which are fertilised are retained until harvest.

Chapter 5.

Flower Structure and Pollen Transfer

Objectives

Previous trials have identified pollination as being a limiting factor for seed production in apetalous lines, apparently largely due to increased sideworking of flowers by bees. However, in some lines there was a high incidence of aborted pods which could not be explained alone from the observations made of bee behaviour.

It was quite common in the apetalous A lines for the style not to be fully extended but to be caught in the tip of the sepals with the stigma remaining covered. When this occurred it was highly unlikely that pollination would be able to take place before the stigma became unreceptive. The incidence of 'caught styles' appeared to be more common in some apetalous lines than others and may be the reason for higher rates of pod abortion in those lines. Observations of bee visits, reported in the previous chapter, were only made on flowers in which the style had successfully extended and so did not take into account the occurrence of caught styles.

In this set of experiments both the A and B lines used in the 1996/97 trials were grown under hot and cool environments. The effect that temperature had on the size of floral organs was investigated in an attempt to explain why caught styles occurred more commonly in some lines than others.

In addition, to confirm the hypothesis that sideworking of apetalous flowers by bees resulted in fewer pollen grains being deposited on the stigmas of A line plants, pollen loads on the stigmas of A line flowers after pollination by honey bees was also investigated.

Introduction

To appreciate what effect the lack of petals has on flower development and opening it is necessary to understand the pattern of floral organ initiation and development in *B. napus*.

A detailed study of the *B. napus* male fertile variety 'Westar' was conducted by Polowick and Sawhney (1986) using scanning electron microscopy. The authors reported that the first floral organs to be initiated on the floral apex were the sepals, followed by the long stamens located in positions which alternated with the sepals and then the two short stamens. The petal primordia were not visible until after the six stamens were initiated and had begun development.

The sepals provided protection to the developing flower by curving over and enclosing the developing floral organs. The tips of the stamens were always just below the ends of the sepals throughout development, indicating a similar growth rate between them. Most of the early growth of the stamens was dominated by the anthers, and filaments did not begin to elongate until just prior to anthesis. The petals stayed relatively small in comparison with the other organs and began rapid growth just prior to anthesis.

Elongation of the gynoecium proceeded at the same pace as the stamens, so the tip of the gynoecium was generally flush with the tops of the short stamens and there was very little space between them and the curved tips of the sepals.

The conditions under which floral development occurs also has a significant impact on the size of floral organs. Polowick and Sawhney (1987) investigated the influence of temperature on the morphology and size of floral organs in an *Ogura* CMS line of 'Westar'.

The authors found that under a high temperature environment of 28/23 °C (day/night), the filaments of CMS plants developed normally but had shrunken anthers that contained no pollen. Under lower temperatures (18/15°C) there was increased instances of feminisation of anthers, which involved the production of external ovules capable of being fertilised as well as stigmatic surfaces on anthers. The normal line grown under the same conditions did not show any evidence of this occurring.

The size of the various floral organs grown under different temperatures was also examined. Under all temperature conditions the length of sepals, petals and stamens from the normal line were significantly greater than those in the CMS line. In both lines the floral organs were larger when the plants were grown under lower temperatures, however the gynoecium length remained relatively constant. It was suggested that the effect on the size and form of floral organs was the result of temperature affecting the level of endogenous plant growth regulators (PGRs). Support for this was provided where exogenously applied PGRs induced effects similar to those of a temperature regime. It has also been reported that *Ogura* CMS lines have altered endogenous levels of cytokinins and abscisic acid in comparison with normal lines (Singh, 1995), which helps to explain the observed differences.

Polowick and Sawhney (1987) reported that while gynoecium development appeared normal in the CMS line, occasionally it was slightly hampered by the reduction in sepal length. Unless the gynoecium was able to push its way through the tightly overlapping sepals, the end became trapped in the tip of the sepals producing a crooked or caught style. This phenomenon of caught styles was also reported by Ogura (1968) (cited in Polowick and Sawhney, 1987) to occur in *Raphanus*, the original source of *Ogura* CMS.

Methods

Measurements of Floral Parts

Seeds of A and B apetalous lines of 1803, 1806, 2807, 2809 and the petalled line 20894 were sown on May 15, 1997 at the University of Tasmania, Hobart, into a potting mixture consisting of pine bark (70%), sand (20%) and peat (10%). Nutrients in the form of a slow release fertiliser (Osmocote™ 300 g/50 l), ferrous sulphate (25 g/50 l) and trace elements were added to the potting mixture, while nutrients (Hoaglands solution, Hoagland and Broyer, 1936) were applied to the pots weekly. For each A and B line six plastic pots, with a diameter of 14 cm and height of 15 cm, were planted with eight seeds and the seedlings were thinned to two per pot 15 days after sowing.

Eight weeks after sowing most plants had produced 10 full leaves and three pots of each A and B line were transferred into a glasshouse and shadehouse respectively, to provide high and low temperature treatments. The high temperature treatment had an average minimum:maximum temperate of 14:26 °C and the cool treatment 2:17 °C. Each treatment received 12 hours of light with natural light supplemented by incandescent globes.

From each of the temperature treatments 15 flowers from each line were collected at random at anthesis. Measurements were made of the sepals, stamens and gynoecia and it was noted whether or not the style was free of sepals and the stigma exposed.

Pollen Transfer

The number of pollen grains deposited on the stigma of A line flowers by honey bees was counted on flowers from plants grown under the cool temperature

treatment. A small hive of honey bees (5,000-6,000 individuals) was placed in a glasshouse measuring 3 m x 5 m, which was kept at approximately 20°C during the day by an airconditioning system. For three days after the bees were initially placed in the glasshouse male sterile A line plants were introduced so the bees could become accustomed to working canola flowers without picking up any pollen.

The A and B line plants used for the pollen transfer experiment were initially kept in a pollinator-free glasshouse and spaced far enough apart to ensure that pollination through physical contact could not occur.

Five pots of each A and B line plants of a respective line were placed early in the morning in the glasshouse containing the bee hive to allow transfer of pollen from B line to A line flowers. The plants were left in the glasshouse for at least ten hours, and then removed once the bees had returned to their hive just before sunset. Only one line was placed in the pollinating glasshouse on each day, to ensure pollen transfer occurred between the A and B line plants of the same line.

Immediately after removal from the glasshouse twenty recently opened flowers from A line plants were removed and the number of pollen grains on each stigma was counted. The sampled flowers of lines 1803, 2807 and 2809 had no petals, however for line 1806 very few entirely apetalous flowers were produced so most flowers sampled had one petal.

Stigmas were removed from the sampled flowers and prepared by immersion for several seconds in a staining solution consisting of 2 g of methylene blue dissolved in 10 ml of ethanol, made up to 200 ml with distilled water. After staining the stigma was rinsed with a few drops of ethanol then gently rinsed again in distilled water. The pollen grains stained a dark blue colour and stood out clearly on the stigma which remained pale yellow (figure 5.1).

The style was then placed in the end of a pasteur pipette, and examined with a dissecting microscope at 80x magnification. The style could be rotated and the number of pollen grains on the stigma counted.

Figure 5.1. A stigma dissected from an A line flower showing pollen grains after staining with methylene blue solution.

Results

Measurement of Floral Parts

Generally plants grown under the lower temperature treatment produced flowers with larger floral organs in both A and B lines (figure 5.2). In turn B line plants produced larger floral organs than A line plants under both temperature regimes. However the magnitude of the difference between treatments varied between lines and floral organs, and gave some indication as to why some lines produced more flowers with caught styles than others.

The floral organ with the most consistent size over lines and treatments was the gynoecium. Only the A lines of 1803, 2807 and 2809 grown under the high temperature treatment produced significantly shorter gynoecia in comparison with plants grown under the low temperature treatment or their respective B lines.

The flowers of all A lines produced significantly shorter stamens than their respective B lines under both high and low temperature treatments. This was caused not only by smaller anthers but also shorter filaments, with the differences between treatments and A and B lines being almost identical for both long and short stamens (figure 5.2).

The anthers of A line flowers were all similar having a shrunken, desiccated appearance with no evidence of pollen production. However, there were some differences in the morphology of filaments between lines. The A lines of 20894, 1803 and 1806 under both temperature treatments, produced filaments which appeared similar to, though much shorter those of the B line flowers. However the filaments of 2807 and 2809 A lines from flowers grown under lower temperature were similar to those of the infertile anthers in that they were thinner and had a desiccated appearance.

The A line stamens also showed a significant response to temperature, with the A lines of 1803 and 2809 showing the strongest response in producing longer long and short stamens when grown under lower temperatures.

Temperature had a larger effect on sepal size of B line plants than was seen in other floral organs. B line sepals of all lines were significantly shorter from plants grown under high temperature conditions.

Figure 5.2. The effect of High and Low temperature treatments on the size (mm) of the floral organs of A and B line flowers. (Data presented in appendix D).

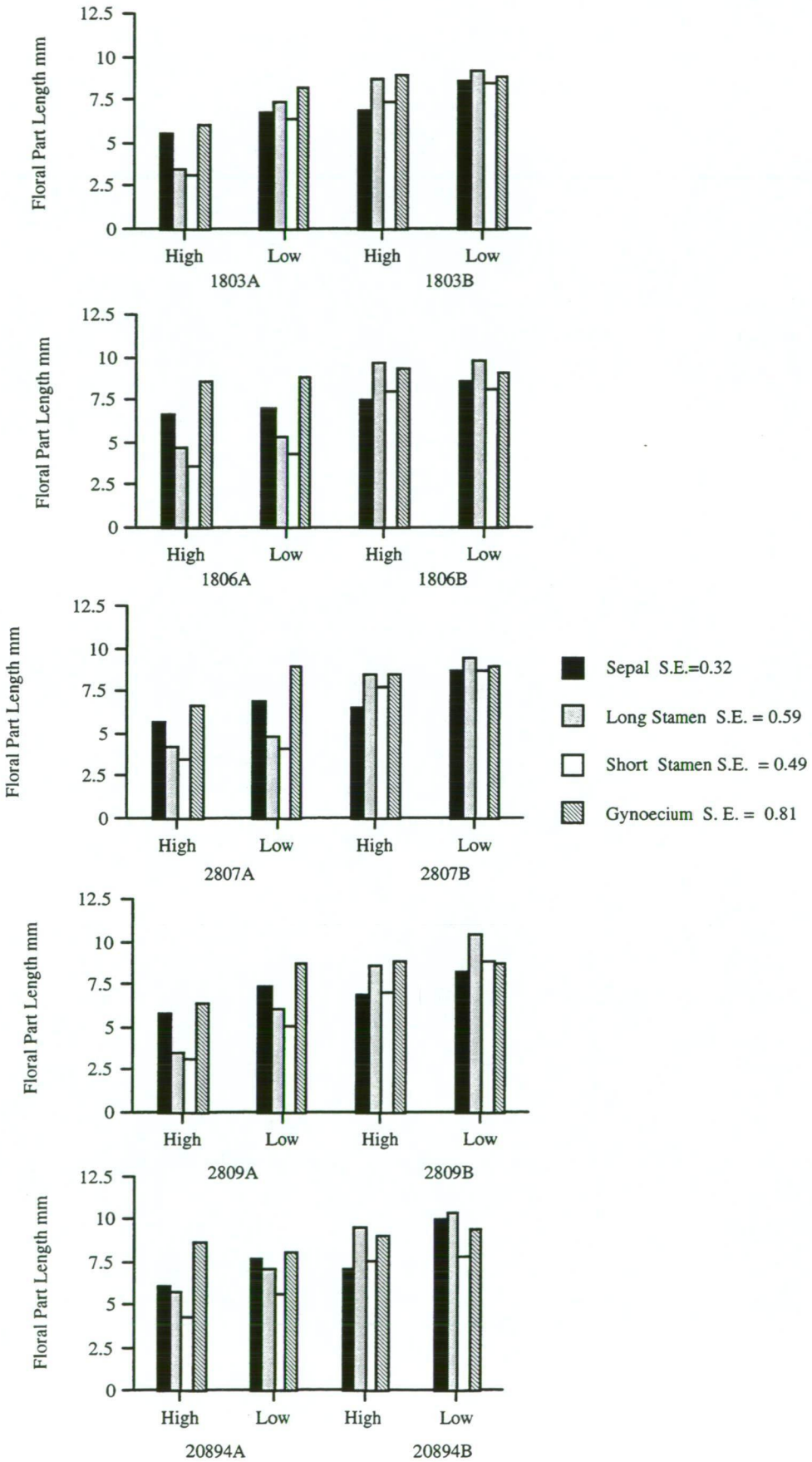

Floral Part Length mm
12.5
10
7.5
5
2.5
0
High
Low
High
Low
1803A
1803B
1806A
1806B
2807A
2807B
2809A
2809B
20894A
20894B
Sepal S.E.=0.32
Long Stamen S.E. = 0.59
Short Stamen S.E. = 0.49
Gynoecium S. E. = 0.81

Table 5.1. The percentage of flowers sampled with caught styles.

	1803		*1806*		*2807*		*2809*		*20894*	
Temperature	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
High	93	0	0	0	47	0	80	0	0	0
Low	0	0	33	0	87	0	67	0	0	0

Figure 5.3. Flowers from 20894 B line plants grown under high temperature (left) and low temperature (right).

Although petal size was not measured, it was obvious that line 20894 produced much larger petals when grown under low temperatures (figure 5.3). Many of the apetalous lines had flowers which produced one to four petals when grown under low temperatures, while remaining completely apetalous under the high temperature treatment. The production of vestigial petals was more pronounced in lines 1806 and 2809, with most flowers produced by 1806 having at least one petal. Several plants of 2809 produced flowers with four petals which were noticeably narrower than those of 20894.

The results presented in table 5.1 indicate that some lines had a higher incidence of caught styles than others. This was caused by the sepals failing to open sufficiently

in order to release the style (figure 5.4b). This was also influenced by the temperature under which the plants were grown. When grown under high temperature 93% of flowers sampled from the A line of 1803 had caught styles, while plants grown at low temperature produced flowers which all opened successfully. There was also a large difference between temperature treatments for the A lines of 2807 and 1806, but with more caught styles at lower temperature.

a) b)

Figure 5.4. Flowers from line 2807 grown under high temperature. (a) B line flowers were able to open successfully without petals, (b) while many A line flowers had caught styles.

That all apetalous B line flowers opened successfully indicated that petals are not essential for flower opening, and that shorter sepals do not appear to impede the growth of the style. Therefore the reason for caught styles occurring in A line flowers would appear to be related to the restricted development of both long and short stamens. Generally the apetalous A lines and treatments which had the largest difference between the length of the gynoecium and long and short stamens had the greatest number of flowers with caught styles.

Pollen Transfer

The absence of petals resulted in significantly less pollen being deposited on the stigmatic surface of the apetalous flowers (table 5.2), presumably due to the greater tendency of honey bees to sidework apetalous flowers. Even the presence of one petal, as occurred for line 1806, resulted in more pollen being deposited in comparison with other lines.

The results show that the number of pollen grains deposited on the stigmas of fully apetalous flowers was very similar between the lines. All of the flowers sampled in this experiment had styles that were fully extended and exposed. In addition, several stigmas were examined in which the style had been caught, and they did not have any pollen present on the stigmatic surface.

Table 5.2. The number of pollen grains on the stigmas of A line flowers from plants grown under the low temperature treatment.

A Line	*Number of Pollen Grains/Stigma*
1803	22.1
1806	41.8
2807	26.4
2809	26.0
20894	70.8
lsd(0.05)	*14.08*

Discussion

The results produced from this limited set of experiments assisted in explaining many of the observations made in previous field experiments.

The high incidence of caught styles in some apetalous A lines would be a major limitation for yield as these flowers are unlikely to be pollinated and produce seed. The fact that this occurred more frequently in the A lines of 2807 and 2809 offers some explanation as to why these lines produced the highest number of aborted pods in previous field trials, where observations suggested that flowers with caught styles were more common in these lines.

The ability of A line flowers to open successfully was influenced by temperature and its effects on the size and development of floral organs. This was most evident in the A line of 1803 which produced a large number of flowers with caught styles when grown under high temperature, but none when grown under cool temperature. The reason for flowers opening successfully when grown under cool temperatures appeared to be due to the larger stamens produced under these conditions, helping to force the sepals open and making pollination possible.

As apetalous B line plants have fully functional stamens the development of these organs continued normally under all conditions. The rapid development of filaments just prior to anthesis as reported by Polowick and Sawhney (1986) appeared to play a major role in forcing open the sepals in B line flowers. Figure 5.5a shows a flower of an apetalous B line (1806), and clearly demonstrates how the large and well developed stamens prevent the style from becoming caught in the sepals. In contrast the stamens of the A line flower of 1806, (figure 5.5b and c) are considerably shorter than the style and there is a much greater likelihood of the style becoming caught.

When the sepals were removed from the A line flower (figure 5.5c) it is apparent that the way in which the short stamen grew out at a wide angle from the style was the main reason that the sepals were forced open and the flower was able to open successfully.

a) b) c)

Figure 5.5. (a) Apetalous B line flower and (b) A line flower. (c) The A line flower with the sepals and one short stamen removed. All flowers sampled from line 1806 grown under high temperature.

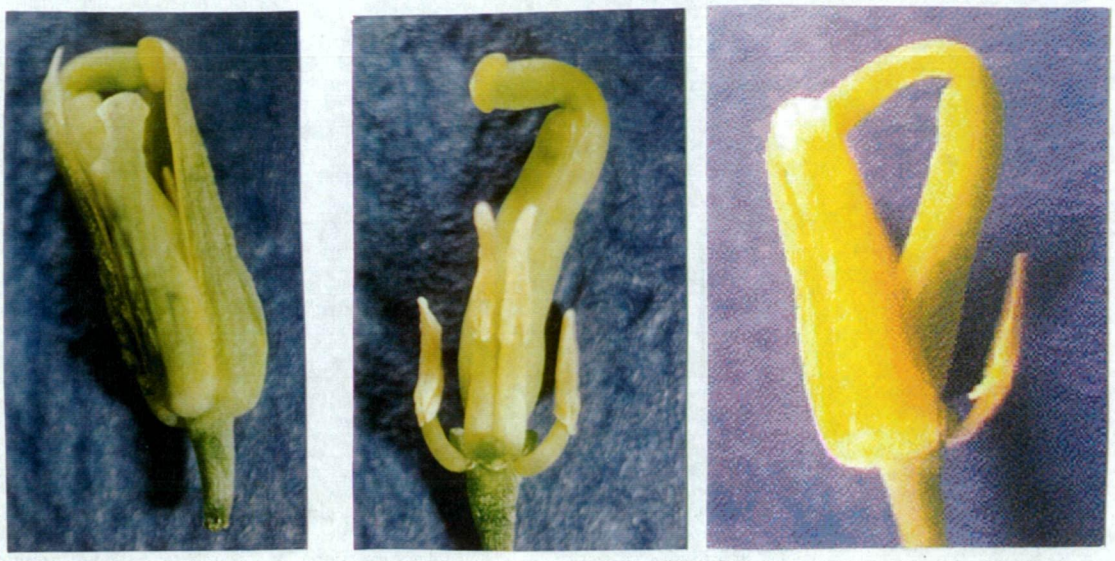
The poorly developed stamens produced by A line flowers of 2807 and 2809 grown under low temperatures, and the growth habit of the short stamens under high temperatures resulted in a high occurrence of caught styles in these lines. This was further affected by the growth of the gynoecium which varied under the different temperature treatments. In the A line of 2807 47% of flowers sampled from plants grown under high temperatures had caught styles, while flowers from plants grown under low temperatures produced significantly longer styles which appeared to be responsible for the increased occurrence of caught styles (87%).

The length of the stamens did not always adequately indicate the ability of these organs to force sepals open and release the style as occurred for line 1803. This ability was reflected more in the thickness of the filament and the angle which it formed with the style, particularly in the case of the short stamens as previously described for line 1806.

Figure 5.6a shows an A line flower from 2807 in which the style was caught. When the sepals were removed (figure 5.6b) it can be seen that the stamens have a similar appearance to those of 1806 (figure 5.5b), however the short stamens are more upright and are positioned closer to the style. As a result there was no physical force pushing the sepals apart, therefore as the style continued to grow it became caught in the tips of the sepals. This problem was further compounded when line 2807 was grown under the low temperature treatment. As previously discussed the stamens were even more poorly developed in this treatment, which combined with a longer style greatly increased the likelihood of stamens becoming caught (figure 5.6c).

In the A line petalled flowers of 20894, poorly developed stamens did not pose a problem in regards to flower opening, as the petals acted as a backup mechanism and forced the sepals open in the absence of well developed stamens.

Change in floral size was suggested by Polowick and Sawhney (1987) to be caused by altered balance of endogenous plant growth regulators as a result of different temperatures. The fact that lines in this trial responded differently to temperature treatments possibly reflects the different genetic backgrounds of the lines investigated.



a) b) c)

Figure 5.6. All flowers are from line 2807. (a) A line flower sampled from a plant grown under high temperature with a caught style, (b) the same flower with sepals removed and (c) an A line flower from a plant grown under low temperature.

The results do indicate that a well-structured flower would represent a significant advantage in seed production. Clearly, it would be important to select A line plants which produce well developed stamens under conditions which would be expected in the field. The length of the gynoecium does not appear to be so crucial if stamens are able to successfully force open the sepals, as occurred in the A line of 1806. However, having stamens which are a similar length to the gynoecium would appear to be a more reliable solution to successful flower opening.

If the flower of an apetalous A line did manage to successfully present a stigma for pollination the results from the pollen transfer experiment indicate that it would have fewer pollen grains deposited on it by honey bees compared with a petalled A line flower. The difference in the number of pollen grains deposited on petalled and apetalous flowers was reflected in the number of seeds/pod produced by the respective lines in previous field trials, and it was clearly evident that pollination

was a limiting step in seed production for the apetalous lines. If a 40% germination rate was assumed for pollen grains deposited on stigmas in this trial (Rao *et al.*, 1992), not all ovules of the apetalous plants would have been fertilised. In contrast, there would have been sufficient pollen for fertilisation of all the ovules in the petalled flowers.

The increased incidence of sideworking bees would appear to be the reason for the lower number of pollen grains deposited on the stigmas of apetalous flowers. Sideworking behaviour by bees may reduce the chance of successful pollination in several ways. If the B line flower is sideworked then it would be expected that the bee would have less pollen adhering to its body than from a visit when direct contact is made with the anthers. In subsequent visits to A line flowers there would be less pollen on the bee to begin with, as well as a reduced chance of the bee making contact with the stigma of apetalous flowers.

The conditions under which pollination took place in this experiment were probably more conducive for pollination than would occur in the field. There was a high concentration of bees with no other available food source and the distance between the A and B line plants was less than one metre. It is highly likely that under field conditions the amount of pollen deposited on stigmas would be less, especially on plants positioned further from the pollen source. When this in taken into consideration, the lack of pollen deposited on male sterile stigmas is clearly a major limitation to apetalous A line seed yields.

Chapter 6.

The Effect of Distance from the Pollen Source on Yield

Objectives

The results from previous trials have shown how CMS hybrid seed production is dependent on the successful transfer of pollen from the male fertile to male sterile plants. Honey bees are the most commonly used pollen vectors in Australia as they are effective pollinators and their social structure enables communities to be directly placed into crops requiring pollination.

As the previous chapter clearly demonstrated, yields obtained from A line plants are highly dependent on the way in which bees interact with flowers to ensure successful pollination. Another factor affecting the success of pollination that has not yet been considered, is the spatial position of A line plants in relation to the pollen donor.

In this chapter the results from two seasons of trials have been examined to determine what effect the distance from the pollen source, in this instance B line plants, had on yield and yield components of A line plants. In addition, a combined analysis of yield and yield components was conducted where possible to investigate seasonal effects.

Introduction

In CMS hybrid seed production systems, once flowering has finished the pollinator rows are usually removed to prevent contamination of A line seed. The area occupied by the pollinator is therefore unproductive in terms of total seed yield and

needs to be minimised for efficient production. However it has also been recognised that yield decreases with increasing distance from the pollen source (Robinson, 1984; Pinnisch *et al.*, 1990), so it is important to determine the optimum ratio of male fertile to male sterile rows which produces the greatest amount of hybrid seed per unit of total land area.

Pinnisch and McVetty (1990) examined the yields produced by *Polima* A lines plants on a row-by-row basis in seed production blocks consisting of thirty rows of A line planted on either side of two, three row plots of pollen parent to give a A:R line ratio of 10:1. While the total yields obtained were quite high, ranging from the equivalent of 3000 kg/ha from the row closest to the pollen parent to 1500kg/ha on the most distant row, the percentage of hybrid seed produced was very low. Only 11% of the seed produced on the most distant row was hybrid seed, while 47% of the seed from the row closest to the pollen source was hybrid. The low levels of hybridity were attributed to a breakdown in the male sterility of the *Polima* A line allowing it to self pollinate.

In subsequent trials McVetty and Scarth *et al.* (1995) used various A:R line ratios to determine the most efficient ratio for hybrid seed production using *Polima* A lines, taking into consideration the area occupied by the R line rows and the level of hybridity required. A ratio of 3A:3R was considered to be economically viable which resulted in a yield of 592 kg/ha with a mean hybridity of 90%.

For hybrid seed production in Tasmania using *Polima* A lines, production blocks are usually planted with a 4:1 A line:R line ratio consisting of 32 rows of A line planted on either side of 8 rows of R line. With the cool summers usually experienced in Tasmania, the *Polima* male sterility rarely breaks down and so a high percentage of hybrid seed is still produced with the larger A:R line ratio.

Renard and Mesquida (1983) cited in Pinnisch and McVetty (1990), recommended a 7:1 A line, R line ratio (14 A line rows : 2 R line rows), for hybrid seed production using *Ogura* winter rape, resulting in a maximum distance between A and R lines of 2.5 metres or 7 A line rows.

With the high degree of stability of the *Ogura* A lines, it may be possible to increase the A:R lines ratio without the need to consider the effect on the hybridity of the seed produced. The most economic A:R line ratio could then be determined solely on a yield basis. The additional problem of poor pod and seed set associated with apetalous flowers may mean, however, that more males will be needed than with normal petalled flowers.

Bee Behaviour

The decline of A line yields with increasing distance from the pollen source is related to the behaviour of the insect vector used for pollen transfer. Higher yields obtained on rows closest to the pollen source are the result of bees working on a pollinator row being more likely to move to a nearby rather than remote male sterile row. This aspect of bee behaviour was described by Free (1970) who noted that in compatible plants grown in adjacent blocks, most intercrossing will occur near to where the blocks adjoin and will rapidly diminish with distance.

Although honey bees move fairly frequently from one plant to another they tend to have quite small foraging areas. It has been observed that many bees return to the vicinity of plants on which they were originally marked (Free, 1970), which reduces the chance of pollen transfer to distant A line plants. It has also been observed that in orchards the presence of tree varieties in discrete rows assist bees in orientating themselves and so they are more likely to return to a specific variety (Free, 1966a,

cited in Free, 1970). A similar occurrence was reported in rapeseed by Pierre (1995) who observed that bees consistently returned to either a petalled or an apetalous line.

As well as the problem of bees being less likely to visit outer rows after being dusted with pollen there is the question of pollen carryover, that is how many male sterile flowers a bee is able to pollinate after a visit to a male fertile flower.

Pollen carryover was investigated by Cresswell *et al.* (1995) through the use of a fluorescent dye powder applied to the anthers of oilseed rape plants followed by the examination of flowers subsequently visited by honey bees. As previously discussed it was observed that bees most frequently flew short distances between visits to individual flowers and most often landed on adjacent plants. Less frequently bees made longer flights which skipped plants, though in both instances bees probed relatively few of the available flowers on each plant before moving to the next.

Most of the dye was deposited on the first few flowers visited and the deposition distributions were similar to the leptokurtic decay curves that are typically obtained in studies of this nature (Cresswell *et al.*, 1995). In this study the mean number of flowers visited in which dye was detected after initial dye collection was five flowers. However, it was recognised that the situation may be different in male sterile plants as the absence of pollen in receipt flowers can extend pollen carryover to more flowers (Price and Waser, 1982 cited in Cresswell *et al.*, 1995).

Therefore, most of the pollen was deposited on the closest neighbours indicating why yields may be higher in male sterile rows closest to the pollen source. The chances of a bee with pollen visiting a flower is less the further the flower is situated from the pollen source, and so yields decline.

Methods

In the trials conducted in 1995/96 and 1996/97 (see Methods, Chapter 3, page 61 and Chapter 4, page 84) the A line rows were harvested individually, allowing the relationships between the distance from the pollinator row and the resulting yield and individual seed weight to be examined. Regression analysis was conducted on these results.

In the 1995/96 trials an analysis was performed using more detailed yield component data from lines 1803, 1806 and 20894. The analysis examined the effect that distance from the pollen source had on yield components.

Results

Subsample Data

The subsample data for yield components were taken from the 1995/96 trials, and comprised of the row closest to the pollen source (row 1), and then every second row. While the data do not show statistically significant differences they do indicate yield component trends which help to explain why in most instances yield declined with increasing distance from the pollen source (figure 6.2). The amount of variation in yield which could be explained with distance from the pollen source varied between lines (r^2 values, table 6.1).

Figure 6.1 displays how the yield components were affected with increasing row number. As demonstrated earlier the petalled line 20894 (Chapters 3 and 4) produced many more seeds/pod than the apetalous lines (figure 6.1a), and the number of seeds/pod remained at a similar level in rows one to eleven. In contrast

lines 1803 and 1806 had a reduction in the number of seeds/pod. This occurred after row one in 1803 and row three in 1806.

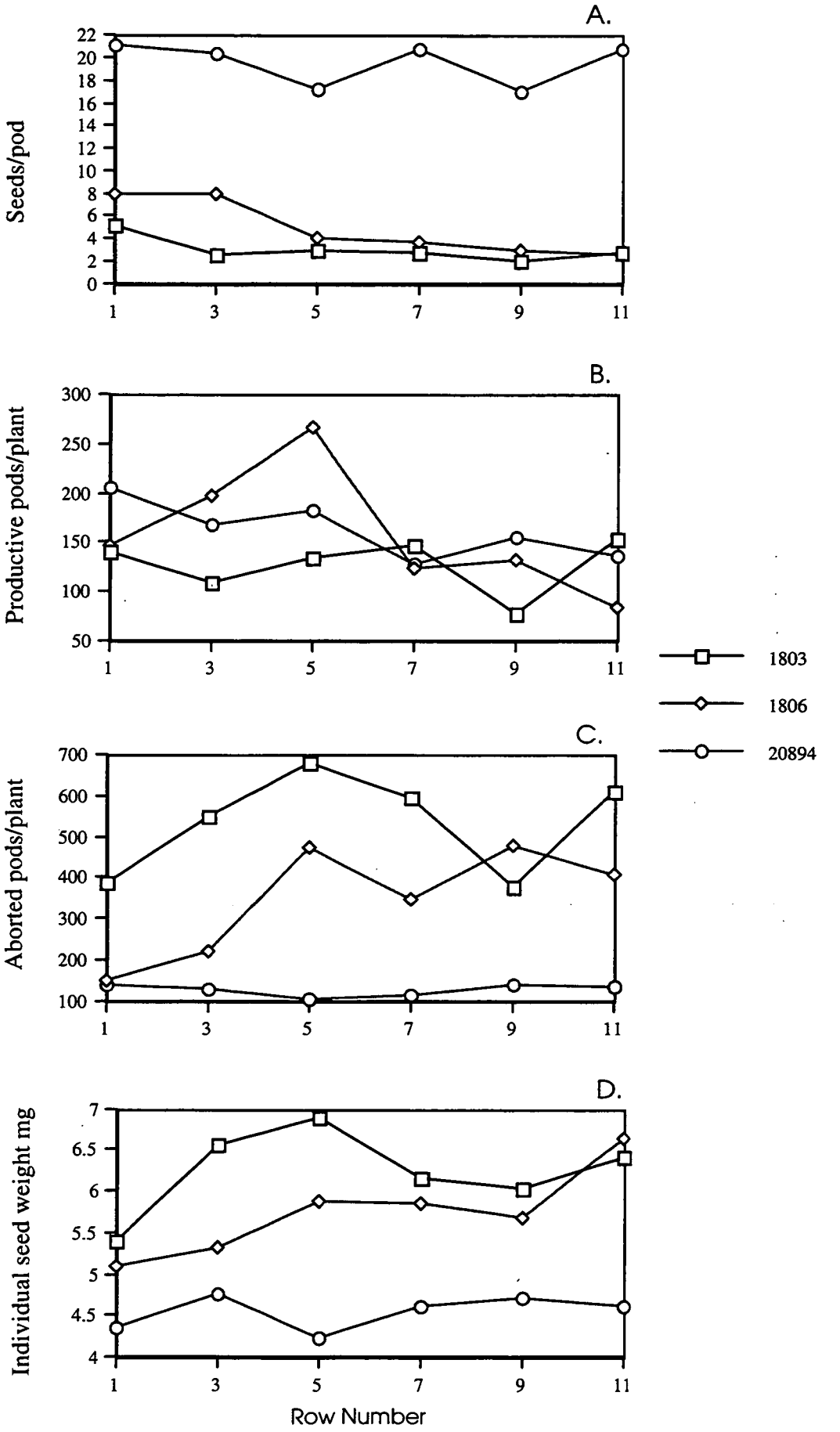
The number of productive pods/plant (figure 6.1b) produced by 20894 declined quite steadily over the rows sampled. The response in the apetalous lines for this yield component was more erratic, with 1806 showing a increase and then a decline while levels were less variable for line 1803.

The apetalous lines, especially 1806, appeared to compensate for the low number of seeds/pod by producing more flowers/plant in the outer rows, as indicated by the large number of aborted pods/plant (figure 6.1c), whereas in contrast 20894 maintained a low level of aborted pods over all rows sampled.

Another compensatory mechanism of the apetalous lines was to increase the individual weight of seeds on the outer rows, once again more so for line 1806. These rows carried a reduced seed load and therefore had more assimilate available per seed (figure 6.1d). Line 1803 produced heavier seed in row three compared to row one, and then maintained a similar weight for the remaining rows, while line 20894 showed no evidence of compensatory seed growth in this set of data.

Figure 6.1. Change in yield components with row number. Row 1 being the closest to the pollen source The data for each row is the average of five plants harvested from the 1995/96 trials; a) the number of seeds/pod, b) the number of pods containing seeds/plant, c) the number of aborted pods estimated from blind pedicles and pods not containing any seeds, d) individual seed weight.

Change of Yield Components with Row Number



Plot Data - Regression Analysis

Regression analysis was performed on the yield and individual seed weight data obtained from all rows within plots (table 6.1), and confirmed the spatial responsiveness of seed yield quantified with the subsample data. The data from the trials conducted in 1996/97 were also subjected to the same analysis.

All of the apetalous lines shared highly significant linear regressions for yield versus row number ($P<0.01$) in the 1995/96 and 1996/97 trials, indicating that row yields decreased the further each row was situated from the pollen source. For the petalled line 20894, the regression for yield was significant at the $P<0.05$ level in 1995/96 but was not significant in 1996/97 although a yield decrease was implied.

While the linear regression analysis for yield was highly significant in the apetalous lines, the amount of variation in yield that this factor accounted for varied between 14 and 51% (r^2 values, table 6.1a). Apart from line 1806 in the 1995/96 season, higher r^2 values were obtained when a logarithmic model was used to fit regression curves (table 6.1a, figures 6.2 and 6.3). The size of the increase in r^2 values varied between lines, but overall the logarithmic model appeared to fit the data more accurately as it took into account the higher yields obtained from the rows closest to the pollen source which was followed by a more gradual decline over the more distant rows (for example, figure 6.3c).

All of the lines from the 1996/97 trials had higher yields for rows eight and nine (figure 6.3), this being especially pronounced for the petalled variety 20894 (figure 6.3i). This was caused by the distance between these rows being a tractor tyre width (0.3 metres), rather than the normal row spacing of 0.15 metres, providing plants in these two rows with more space and resources. This effect was not as evident for the data from 1995/96 in which overall yield were lower, and conditions were not as favourable for compensatory growth.

Table 6.1. (a) Plot yield data (b) and individual seed weight data for 1995/96 and 1996/97 trials. Linear regression equation parameters for seed yield and individual seed weight against row number (row 1 being closest to the pollen source) are also presented. Values for r^2(log) are also presented for logarithmic regressions. Regression equations are contained in appendix E.

a) Yield

Year	*Line*	*Mean Plot Yield kg/ha*	*Y-Intercept*	*Regression Coefficient*	r^2	r^2 *(log)*
1995/96	*1803*	837.3	1391.4	-88.7	0.29 ***	0.43
	1806	872.7	1205.1	-55.1	0.38 ***	0.25
	2807	863.5	1505.8	-106.3	0.35 ***	0.52
	20894	2996.7	4229.3	-167.5	0.19 *	0.22
1996/97	*1803*	1773.8	2351.1	-72.2	0.21 ***	0.24
	1806	1395.9	2574.7	-147.4	0.51 ***	0.65
	2807	529.9	874.5	-42.6	0.14 **	0.18
	2809	1288.1	2149.1	-107.6	0.36 ***	0.53
	20894	3644.5	4179.2	-67.3	0.04	0.08

b) Individual Seed Weight

Year	*Line*	*Mean Individual Seed wt.*	*Y-Intercept*	*Regression Coefficient*	r^2	
1995/96	*1803*	6.58	6.50	0.02	0.06	
	1806	5.98	5.27	0.10	0.56	***
	2807	5.38	5.99	0.07	0.24	**
	20894	4.34	4.28	0.01	0.07	
1996/97	*1803*	5.05	4.53	0.06	0.39	***
	1806	5.22	4.88	0.04	0.55	***
	2807	4.64	4.75	-0.01	0.01	
	2809	5.49	5.31	0.02	0.07	*
	20894	3.75	3.42	0.04	0.42	***

*,**,*** Significant at P=0.05, 0.01 and 0.001, respectively.

The r^2 values for yield and the level of significance were affected by the degree to which lines were able to compensate in the yield components not affected by distance from the pollen source, namely extra potential pods and individual seed weight. The 1996/97 season appeared to be more favourable for yield as all lines apart from 2807 produced higher yields than in 1995/96 (table 6.1a). In the 1996/97

season more lines produced heavier individual seed weights on the outer rows as indicated by the significant linear regression relationships for individual seed weight versus row number in all lines except 2807 (table 6.1b, figures 6.3). In the 1995/96 season only lines 1806 and 2807 showed this effect (figure 6.2).

The effect which compensatory growth had on yield was controlled by the total number of seeds set by the respective lines. The petalled line 20894 had the greatest capacity for seed weight compensation as it produced more seeds than the apetalous lines. Under the more favourable conditions experienced in 1996/97, 20894 was able to increase individual seed weight to the extent that yield decline with increasing distance from the pollen source was not significant (figures 6.3 i and j).

In the 1996/97 trials, line 1803 also shared a significant linear relationship for individual seed weight as did line 1806. With more seeds/pod produced by line 1803 the effect had more influence in reducing the r^2 value for yield than in 1806, however not to the same extent as 20894.

The ability to increase individual seed weight differed between lines and appeared to be associated with the number of aborted pods. In the 1996/97 trials, lines 20894, 1803 and 1806 had significantly fewer aborted pods/plant than lines 2807 and 2809 (table 4.2, page 127). The lines with the least aborted pods/plant had very highly significant linear regressions between individual seed weight and row number (table 6.1). The relationship was not significant for lines 2807 and 2809, or significant only at the $P<0.05$ level. This difference was probably related to the amount of compensatory flowering by these lines, caused by pollination problems as previously discussed in chapter 4. The extra flowers and branches produced by lines 2807 and

2809 may have resulted in lower levels of assimilate being available to developing seeds.

In order to eliminate the effect of extra space between drill runs, which caused the rows on either side to yield more than expected given the trends on either side, the yield of these rows was adjusted. Adjusted yields were calculated by assuming that those two rows, one on either side of the drill run, had in effect the area of 1.5 rows, so yield of these rows was reduced by a third.

Figure 6.2. Linear regression plots for yield (a, c, e, g) and individual seed weight (b, d, f, h) for lines 1803, 1806, 2807 and 20894 respectively in 1995/96 trials. Logarithmic regression equations were also fitted to the yield data.

---------- Linear Regression

——— Logarithmic Regression

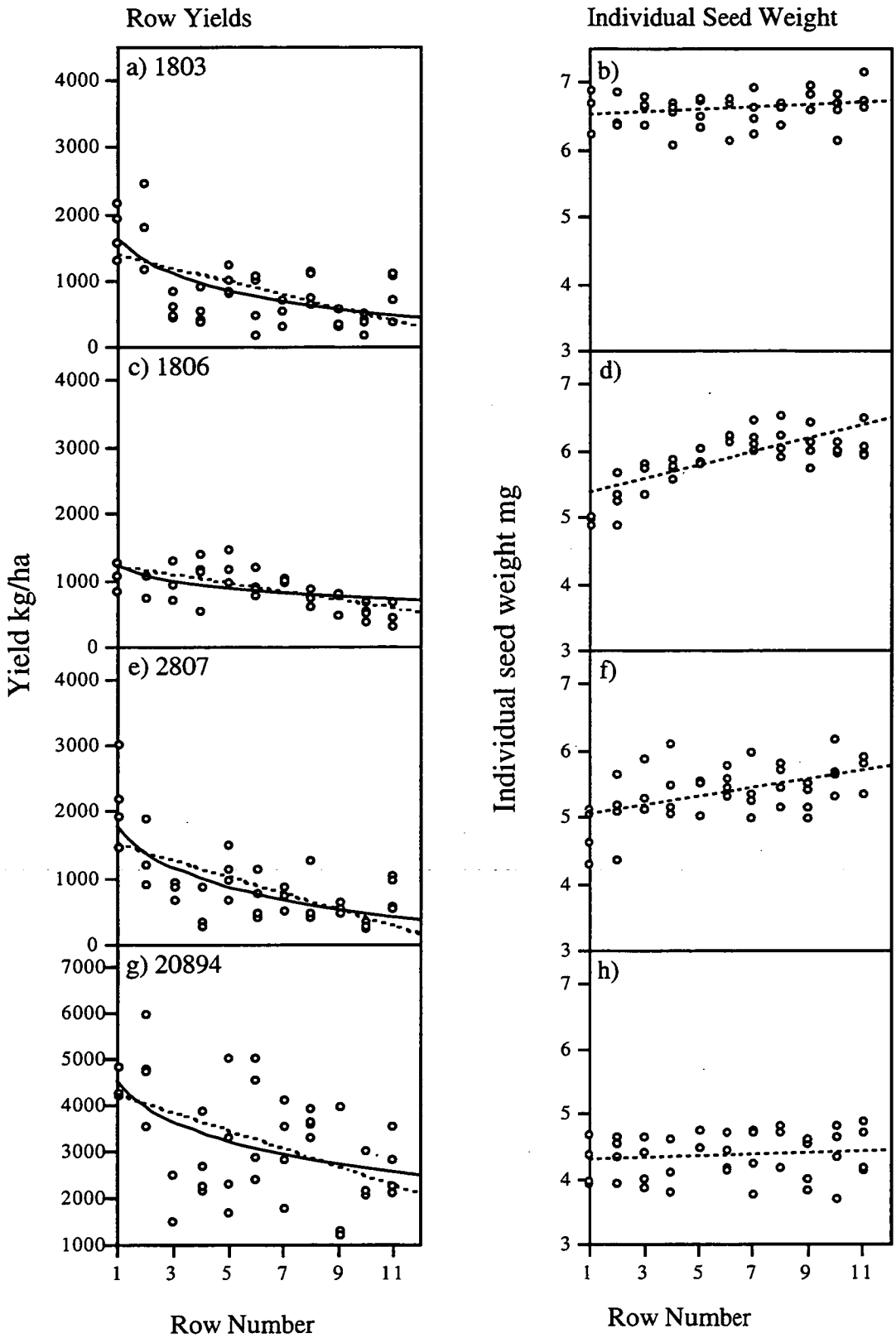
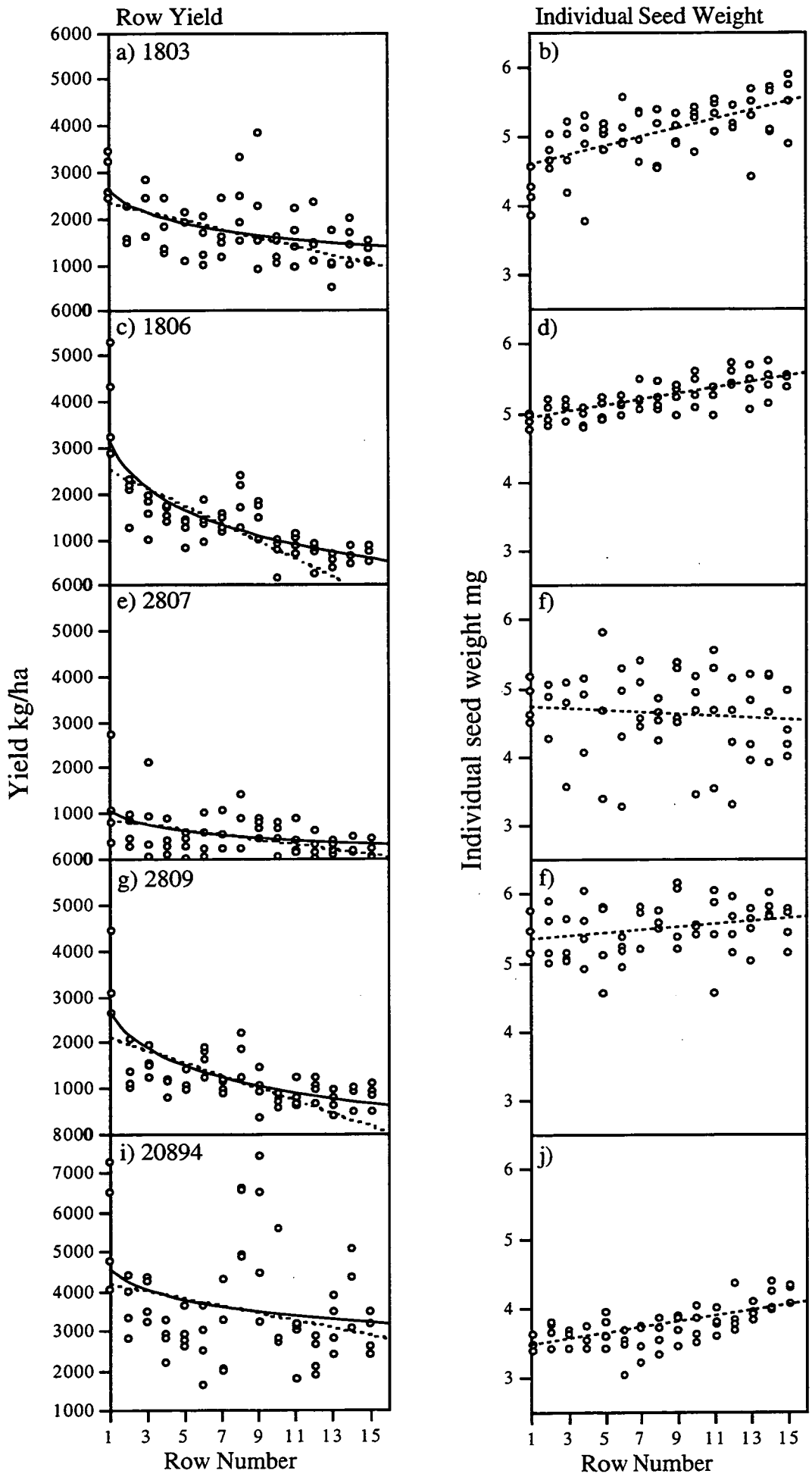

Row Yields
Individual Seed Weight
a) 1803
b)
c) 1806
d)
e) 2807
f)
g) 20894
h)
Yield kg/ha
Individual seed weight mg
Row Number
Row Number

Figure 6.3. Linear regression plots for yield (a, c, e, g, i) and individual seed weight (b, d, f, h, j) for lines 1803, 1806, 2807, 2809 and 20894 respectively in 1996/97 trials. Logarithmic regression equations were also fitted to the yield data.

---------- Linear Regression

——— Logarithmic Regression


Row Yield
Individual Seed Weight
a) 1803
b)
c) 1806
d)
e) 2807
f)
g) 2809
f)
i) 20894
j)
Yield kg/ha
Individual seed weight mg
Row Number
Row Number

Regression Analysis with Adjusted Data

Using adjusted yield data to calculate logarithmic regressions produced higher r^2 values for all lines and demonstrated that by eliminating the effect of the rows with extra ground area a large of amount of yield variation could be explained with the logarithmic model (table 6.2, figures 6.4 and 6.5).

When the number of seeds/m^2 for each row was calculated by dividing yield by individual seed weight, the influence of increasing distance from the pollen source on reducing the number of seeds produced could be clearly seen in the regression analysis (table 6.2, figures 6.4 and 6.5). This eliminated the effect of compensatory growth in individual seed weight and clearly demonstrated that pollination became a limiting factor for seed production in the outer rows of all the lines investigated. Again the logarithmic regression model was able to account for more variation than linear regression. With this analysis the row effect was evident in the petalled line 20894, which in the 1996/97 trials produced a r^2 value of 0.31 for seed numbers, while a r^2 value of 0.19 was obtained for adjusted yield.

Logarithmic regressions for the number of seed/m^2 for line 1806 in the 1996/97 trials produced the highest r^2 values of all lines (table 6.2, figure 6.5d). This line was the latest flowering and obviously received less pollen from other plots as a result.

Table 6.2. r^2 values for linear and logarithmic regression of adjusted yield and the number of seeds/row on row number. (Regression equations are contained in appendix C)

		Linear Regression r^2		*Logarithmic Regression r^2*	
Year	*Line*	*Adjusted Yield*	*Seed Nos.*	*Adjusted Yield*	*Seed Nos.*
1995/96	*1803*	0.28***	0.34***	0.44	0.50
	1806	0.39***	0.53***	0.31	0.48
	2807	0.35***	0.38***	0.53	0.56
	20894	0.21**	0.25**	0.29	0.34
1996/97	*1803*	0.28***	0.39***	0.37	0.53
	1806	0.54***	0.55***	0.72	0.74
	2807	0.16**	0.16**	0.21	0.21
	2809	0.37***	0.38***	0.56	0.58
	20894	0.09*	0.19**	0.19	0.31

*,**,*** Significant at P=0.05, 0.01 and 0.001, respectively.

Figure 6.4. Linear regression plots for adjusted yield (a, c, e, g) and seed numbers/m^2 x 10^{-3} (b, d, f, h) for lines 1803, 1806, 2807 and 20894 respectively in 1995/96 trials. Logarithmic regression equations were also fitted to the yield data.

---------- Linear Regression

——— Logarithmic Regression

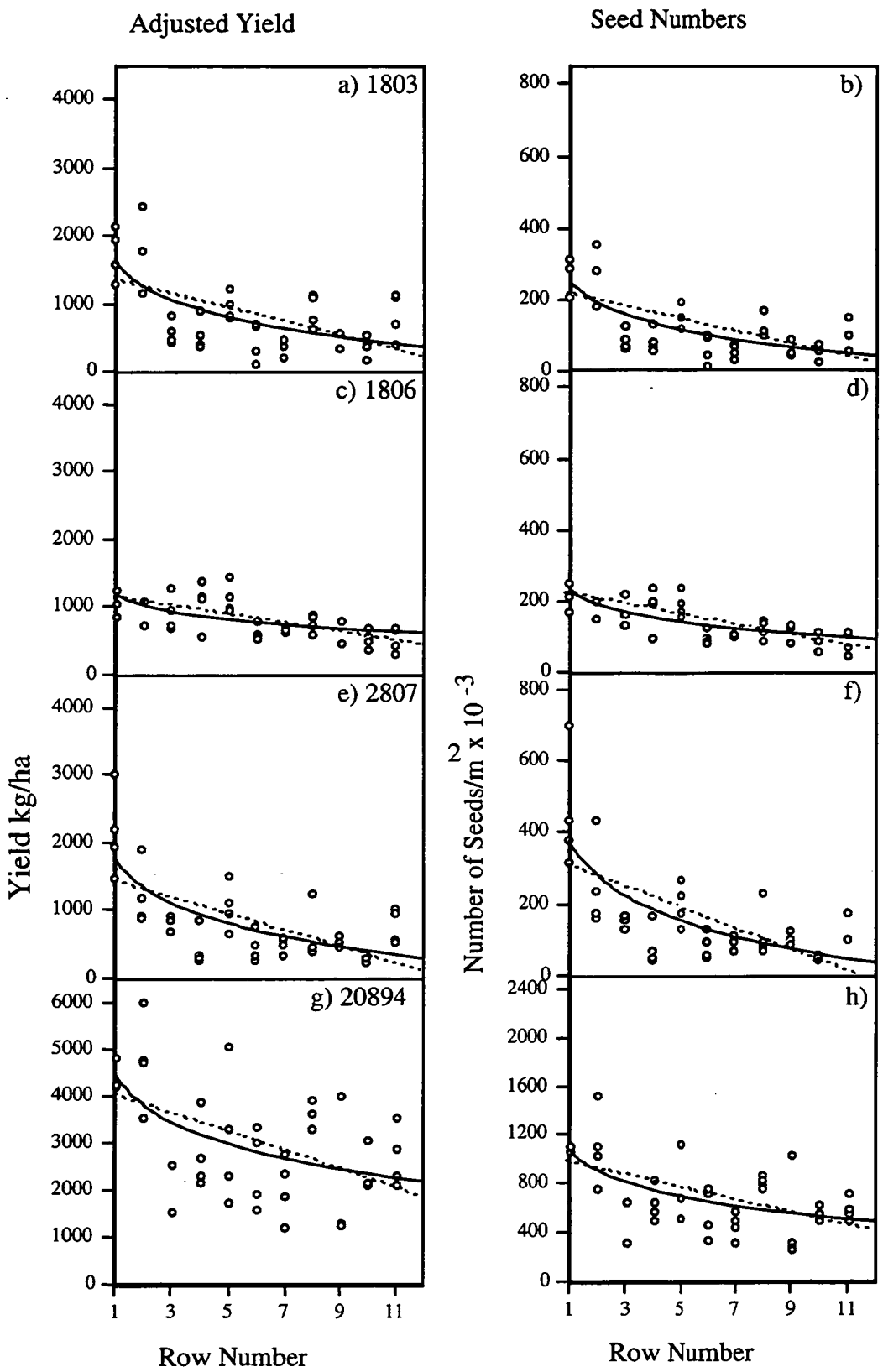
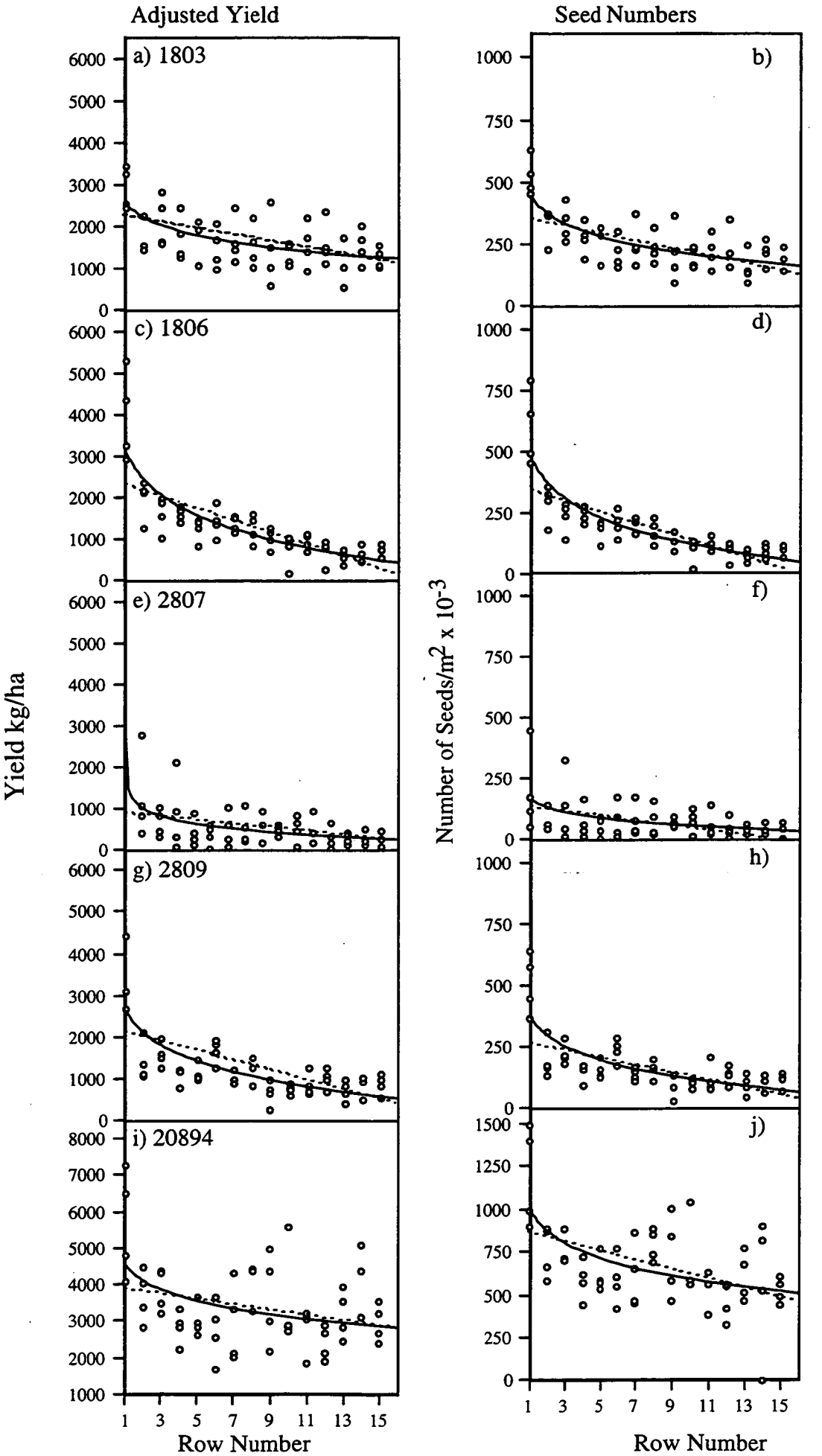

Adjusted Yield
Seed Numbers
a) 1803
b)
c) 1806
d)
e) 2807
f)
g) 20894
h)
Yield kg/ha
Number of Seeds/m 2 x 10 -3
Row Number
Row Number

Figure 6.5. Linear regression plots for adjusted yield (a, c, e, g, i) and seed numbers/m^2 x 10^{-3} (b, d, f, h, j) for lines 1803, 1806, 2807, 2809 and 20894 respectively in 1996/97 trials. Logarithmic regression equations were also fitted to the yield data.

---------- Linear Regression

——— Logarithmic Regression


Adjusted Yield
Seed Numbers
a) 1803
b)
c) 1806
d)
e) 2807
f)
g) 2809
h)
i) 20894
j)
Yield kg/ha
Number of Seeds/m^2 x 10^{-3}
Row Number
Row Number

Logarithmic Regression Models and Combined Season Analysis

To allow a comparison to be made of the effects of different seasons on yield and yield components of the various lines, a combined analysis of variance was conducted where possible, of yield and yield components (table 6.3).

The logarithmic regression models of row versus seed numbers/m^2 discussed earlier in this chapter were used to construct the graphs presented in figure 6.6. This enabled the effect of the environmental conditions on seed set in the 95/96 and 96/97 seasons to be investigated.

As discussed in Chapter 2, all lines produced low yields in the adverse conditions of the 94/95 season. More favourable conditions experienced in the 95/96 and 96/97 seasons, as well as better crop management in regards to nutrition and irrigation, saw significant yield increases for all lines. Generally yields were ranked similarly in each season with 20894 by far the highest, followed by 1803 and 1806, with 2807 generally by far the lowest. The overall poor performance of 2807 was due to this line setting fewer productive pods than the other apetalous lines.

As discussed in Chapter 4, the number of productive pods/m^2 in the 96/97 season estimated from the subsampled plants overestimated this yield component. The results do suggest that lines 1803, 1806 and the petalled line 20894, which all had significantly higher yields than in the previous season, produced more productive pods, together with more seeds/pod resulting in higher yields.

Over the 95/96 and 96/97 seasons the number of aborted pods produced by lines 1803, 1806, 20894 remained relatively constant. However, line 2807 produced significantly more aborted pods in the 96/97 season, when it yielded the lowest of the two seasons.

Table 6.3. Combined yield and yield component analysis for hybrid seed production trials, 94/95, 95/96 and 96/97 seasons.

Yield kg/ha	*94/95*	*95/96*	*96/97*	*Means*
1803	424	837	1773	1012
1806	586	872	1395	952
2807	97	863	529	497
*20894**	1473	2996	3544	2672
		lsd(0.05)= 418		*lsd(0.05)= 241*
1000 Seed Wt gm				
1803	6.01	6.6	5.05	5.88
1806	4.75	5.98	5.22	5.32
2807	4.97	5.4	4.64	5.00
*20894**	3.83	4.37	3.75	3.98
		lsd(0.05)= 0.32		*lsd(0.05)= 0.19*
Productive Pods/m²				
1803		3853	18444	11148
1806		3077	12923	8000
2807		4060	7192	5626
*20894**		3432	10112	6772
		lsd(0.05)=3412		*lsd(0.05)=1896*
Aborted Pods/m²				
1803		16837	16760	16799
1806		11599	13803	12702
2807		17302	28808	23055
*20894**		2297	2118	2208
		lsd(0.05)= 6840		*lsd(0.05)= 3311*
Seeds/Pod				
1803		3.1	10.2	6.7
1806		5.1	7.5	6.3
2807		4	6.7	5.4
*20894**		19.9	21.9	20.9
		lsd(0.05)=2.09		*lsd(0.05)=1.25*

For the seasons in which the apetalous lines produced the lowest yields, the logarithmic regression models of seed numbers/m^2 versus row number were distinctly different (figure 6.6). Examination of the regression models for each season revealed more information than from a combined analysis, and demonstrated a large environmental influence on the apetalous lines in determining seed set. There were also difficulties is combining data from different seasons for regression analysis, as there were a different number of rows of A lines grown in each season. For these reasons a combined regression analysis was not conducted.

By far the greatest influence on the yield of the apetalous lines was their ability to set and/or retain seed. For lines 1803 and 1806 the 96/97 season was more conducive to set seed, as shown by greater numbers of seeds/pod and productive pods. While line 20894 had similar regression models over the two seasons, conditions appeared more favourable in 96/97, which resulted in more productive pods being set, and the highest yield for this line from all the trials conducted.

While better environmental conditions and crop management clearly enhanced seed production in the 96/97 season for line 1803, 1806 and 20894 this was not so for 2807. Against the trend exhibited by the other lines, 2807 produced less seeds/m^2 in 96/97 and hence lower yields. The results of the hand pollination experiment discussed in Chapter 5, indicated that 2807 had the best seed retention ability after hand pollination of all the apetalous lines, which suggests that pollination difficulties were the reason for poor seed set in 96/97.

The experiments conducted on flower structure in Chapter 5 indicated that line 2807 produced many flowers with caught styles. This would appear to by the most obvious reason why this line produced such a low yield in the 96/97 season, and prevented it from performing as well as the other lines.

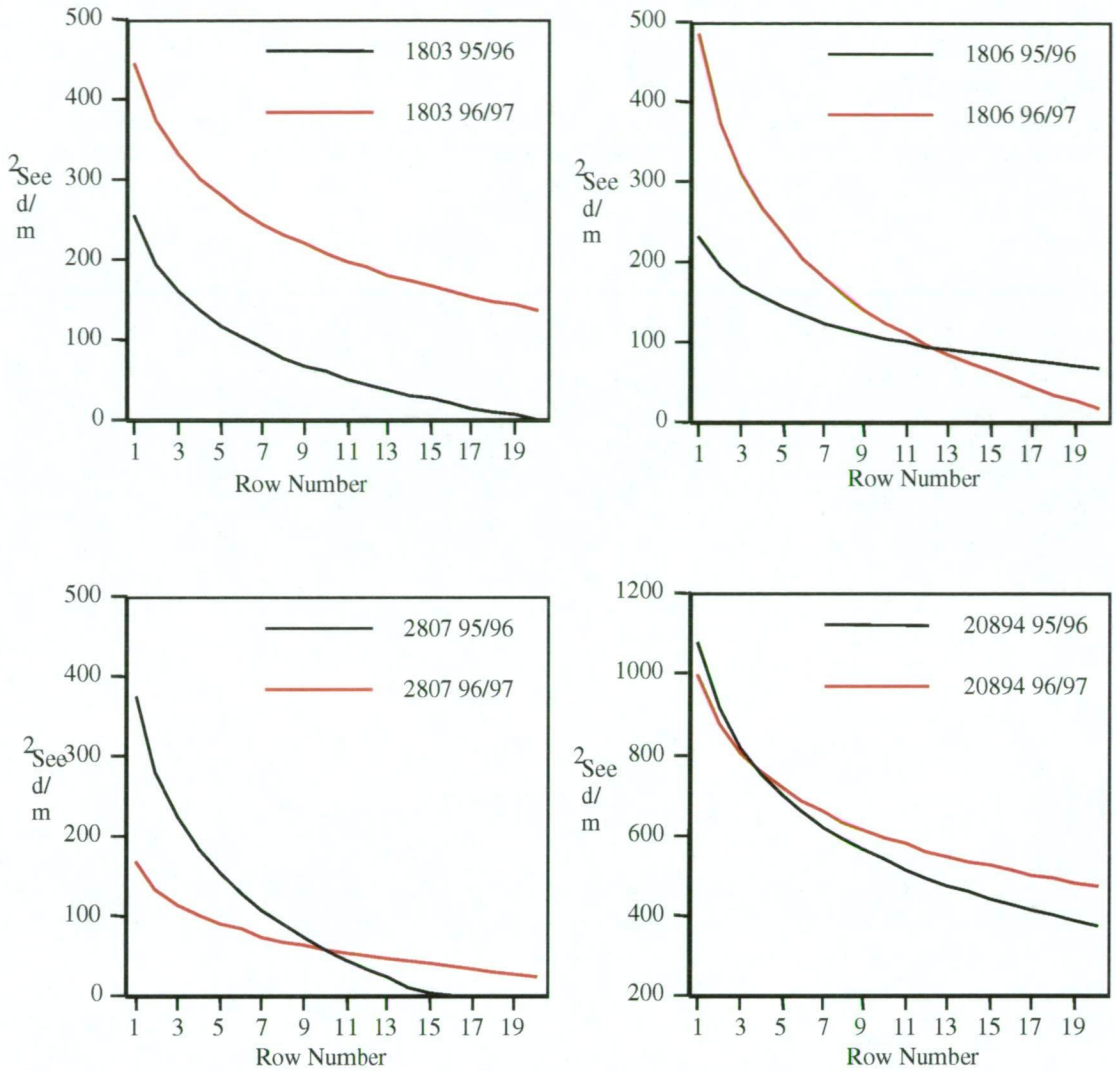


Figure 6.6. Logarithmic decay curves for seed numbers/m^2 on a row basis for lines grown in 1995/96 and 1996/97 seasons. Curve regression equations are contained in appendix E.

Apart from line 2807 the combined analysis demonstrated that while the petalled line also responded to the more favourable pollination conditions, the apetalous lines showed a much more dramatic response, which may make yield budgeting difficult for these lines, if they are used in commercial production.

Discussion

The yield component responsible for the decrease of yield with increasing distance from the pollen source differed between the petalled and apetalous lines. For the petalled line, 20894, lower yields were the result of fewer productive pods being set on the outer rows. Both the apetalous lines for which yield component data were collected behaved similarly, and set less seeds/pod with increasing row number, while the productive pod numbers remained relatively constant. The fact that a significant part of the yield variation could be accounted for by regression with increasing row number from the pollen source indicated that pollination had a major influence on yield, as pollen dispersal by honey bees was shown by Cresswell *et al.* (1995), to have the same distribution pattern. The reason that a higher proportion of yield variation could not be explained by distance from the pollen source was due to compensatory growth in other yield components such as individual seed weight and the number of potential pods. This in turn varied between lines and seasons as the number of seeds/pod and conditions experienced post-flowering changed.

When the compensatory growth of the seed component was eliminated by examining the effect of distance from the pollen source on the numbers of seeds/m^2 produced by each row, the effect of distance from the pollen source became more significant. It was apparent that even the petalled line 20894 was affected by reduced pollination of the outer rows which was not obvious when yield only was examined.

The design of the experiments reported in this chapter would have probably allowed some cross pollination to occur between plots, which is unlikely to occur in field production blocks. Despite this all lines showed significant regression relationships for seeds/m^2 with row number, indicating the strong influence of this factor on pollination. Line 1806, the latest flowering line, produced the highest r^2 value for

adjusted yield in the 1996/97 season when it flowered in isolation after the other lines. During the 1995/96 season, earlier flowering lines of a later sown adjacent trial would have allowed cross pollination to occur, which most likely resulted in the lower r^2 values for that season's data.

The reduction in the number of productive pods and seeds/pod with increasing row number appeared to be caused by bee behaviour, which was further influenced by the effect which apetalous flowers had on pollination. The chance of a flower being visited by a bee dusted with pollen diminishes the further it is situated from the pollen source. As indicated by Cresswell *et al.* (1995), the amount of pollen deposited by bees on flowers follows a logarithmic decay curve. In most situations this model also gave the best estimation of yield reduction as it took into account the higher yields produced on rows immediately adjacent to the pollen source. The logarithmic regression for seeds/m^2 and row number produced even higher r^2 values, as seed numbers are a better indication of pollen dispersal than is yield. This is due to yield being comprised of a number of components, including individual seed weight and seed numbers, while the number of seeds produced is more directly related to pollen dispersal.

The reduced likelihood of a flower being visited by a bee carrying pollen the further it was situated from the pollen source was clearly represented in the petalled line 20894. This line showed a reduction in the number of productive pods with increasing distance from the pollen source. In contrast, the apetalous lines flowered over a longer period of time, which increased the chance of flowers in the outer rows being visited by bees carrying pollen. However these extra flowers were produced on increasingly unproductive secondary and tertiary branches and so were unable to compensate for the fewer pods set on the mainstem and primary branches.

As well as later flowers of apetalous lines being produced on more unproductive branches, the amount of pollen produced by B line plants would most probably be limiting at this stage. B line plants had a more contracted flowering period being self pollinating and without the limitation imposed on flower opening by the absence of functional anthers in the male sterile A lines, they finished flowering while A lines were still producing new growth.

The evidence suggests that apetalous flowers required a greater number of bee visits for the fertilisation of all viable ovules than did the petalled line, and that this was responsible for the large reduction in the number of seeds/pod produced by the apetalous line with increased distance from the pollen source. In chapter 4, the high frequency of bees sideworking apetalous flowers was shown to result in fewer pollen grains being deposited on apetalous stigmas in comparison with petalled flowers (table 5.2, page 112). In the petalled line the yield component most affected by distance was the number of productive pods indicating fewer bees with pollen visited the outer rows. However a similar number of seeds/pod were produced on rows further from the pollen source as on closer rows, which indicates that just one successful bee visit was sufficient to pollinate the majority of fertile ovules present in petalled flowers.

In contrast, for the apetalous lines restricted bee visits resulted in fewer productive pods being produced initially, resulting in increased flower production, which was then compounded by fewer ovules/pod being fertilised. Even in the rows closest to the pollen source the numbers of seeds/pod produced by the apetalous lines was much lower than that of the petalled variety. Even under natural optimal pollination conditions the apetalous lines were unable to match the petalled line in the number of fertilised ovules/pod.

As it appears that the apetalous lines require a greater number of bee visits it is more likely that other factors which may affect bee behaviour such as adverse weather conditions during flowering would have a significant influence on the yield of apetalous lines. This explains why apetalous yields suffer to a greater degree when high temperatures during flowering are encountered. During periods of hot weather, honey bees are more likely to spend time cooling the hive by collecting water than they are gathering nectar and pollen (C. Parker, Cambridge Tasmania, pers. comm.). Therefore, during periods of high temperature not only are the plants affected in terms of a reduced period of stigma receptivity and pollen survival, but also the altered behaviour of bees results in less likelihood of visits by bees being successful.

As a result of the interactions between bees and the different flower types the apetalous lines were affected to a greater degree than were the petalled line as distance from the pollen source was increased, and set less seed if adverse weather conditions were encountered during flowering. The practical implications of this suggest that reduced A line:R line ratios would be needed for apetalous lines to produce economically viable yields. Apetalous A lines must also be 100% sterile for high levels of hybridity to be assured which at this stage would restrict the use of apetalous flowers to the *Ogura* CMS seed production systems.

Chapter 7.

General Discussion

The purpose of this final chapter is to bring together the results of the three seasons of field trials and glasshouse experiments so that recommendations and suggestions can be made on the future use of apetalous canola lines in hybrid seed production.

The initial aims of this research were to establish whether apetalous hybrid seed can be produced, and whether this then produces a crop with higher yields, as a result of decreased solar radiation reflectance and adsorption by the flower canopy. Research conducted by Rao *et al.* (1991) and Fray *et al.* (1996) did establish that higher levels of solar radiation penetrated into the plant canopy as a result of the apetalous character in pure or open pollinated lines, but it has not been shown conclusively that this results directly in higher yields. In the work conducted by Rao *et al.* (1991), while the apetalous line used did produce higher yields, it was compared with a canola line of different genetic background, which confounded the results.

The apetalous line used by Fray *et al.* (1996) was of different origin to those used by of Rao *et al.* (1991), and had poor agronomic characteristics. Again isogenic lines, differing only in the apetalous character, were not available so the results were not conclusive in determining the effect of the lack of petals.

The research presented in this thesis was not focussed on establishing whether or not the apetalous character would result in higher yields. This research concentrated more specifically on examining the effect of combining the apetalous character and

cytoplasmic male sterility (CMS) on yield and yield components of the seed crop. The ultimate aim of investigating the apetalous type should be to determine the potential yield advantage of this character. However, this was not established in these experiments, as a restorer line was not available that would enable the production of F_1 seed for testing, and also there were no isogenic lines available to give a definitive comparison between petalled and apetalous hybrids.

The *Ogura* CMS system used in these trials was shown to be a very promising method of producing hybrid seed, once restorer lines are available. There was no evidence of the *Ogura* male sterility breaking down, despite high temperatures being encountered during flowering. Both the apetalous and petalled lines used in these trials contained the *Ogura* cytoplasm, and the high yields produced by the petalled A line 20894, suggested that this CMS system was not associated with a yield penalty.

The initial trials discussed in Chapter 2, which investigated the yield potential of the petalled and apetalous B lines, showed that a large amount of variation exists between the lines in regards to yield and yield components. This was attributed to the genetic differences between the lines which determine final yield. However, the yield components of the apetalous A lines were very similar over all seasons, especially with regard to the high number of aborted pods and low number of seeds/pod. This indicated that the apetalous-CMS combination had a much greater effect on yield and yield components than did differences in their genetic background.

Over all the trials conducted, yields of the apetalous A lines were unable to match the petalled A line yields. In contrast some apetalous B lines yielded as well as the petalled B lines, indicating that they had a similar genetic yield potential. In male fertile plants, high seed yield is favoured by good vegetative growth, followed by

the production of a moderate number of pods which should enable each pod to maintain near the potential number of seeds (Mendham *et al.*, (1981). This theory did not apply to the apetalous A lines used in these trials. Despite good vegetative growth and the retention of leaves during flowering the apetalous A lines were unable to yield as well as would be expected.

The lower yields of the apetalous lines were the result of a small percentage of productive pods being set from potential pod sites, combined with a low number of seeds/pod in those pods which were retained. In response to the reduced seed load the apetalous plants continued to produce more branches and flowers well after the petalled line and self pollinating B lines had finished flowering. Despite this and the production of heavier individual seeds, the apetalous lines were unable to compensate for poor pod and seed set.

Poor pod and seed set was found to be associated with the effect the apetalous flower characteristic had on flower opening, and the behaviour of honey bees visiting the flower. Pollen vectors (honey bees in this situation) were shown to be essential for seed production on A line plants, and the apetalous flower morphology changed the way in which the bees interacted with the flower while collecting nectar. In normal flowers the petals provided a landing platform for visiting bees which greatly increased the likelihood of contact being made with the stigma. In contrast, bees were more likely to land on the side of apetalous flowers, and move around the bottom of the flower to reach the nectaries making contact with the stigma unlikely. The percentage of successful bee visits (85%) made to petalled flowers was similar to that reported by Pierre (1995) to petalled flowers. However, Pierre (1995) observed that 50% of visits made to apetalous flowers were successful, while the results from the current trials indicated that only 15%-35% of bee visits

made to fully apetalous flowers were successful. McVetty *et al.* (1989) reported that the altered morphology of *Polima* A line flowers which had smaller petals than normal varieties, increased the occurrence of sideworking by leaf cutter bees (*Megachile rotunda*), but in contrast to the present results, this behaviour did not result in lower yields.

The investigation of pollen loads made in Chapter 4 of this thesis, demonstrated that significantly fewer pollen grains were deposited on apetalous A line stigmas than occurred on petalled flowers. This was attributed to increased sideworking by bees during flower visits, and appeared to be a major reason for the poor seed and pod set of the apetalous A lines. Even the presence of just one petal significantly increased the pollen load, which may explain why McVetty *et al.* (1989) did not observe any yield reduction on petalled lines which had a higher incidence of sideworking leaf cutter bees.

The apetalous character was not found to have a negative effect on attracting bees, as there were no significant differences in the number of bees observed visiting apetalous and petalled lines.

The response of apetalous A line plants to hand pollination under field conditions confirmed that pollination was a limiting factor for both pod and seed production. All the apetalous lines produced more productive pods from flowers which were hand pollinated, and initially they produced more seeds/pod. In contrast, the petalled line, 20894 showed no response to hand pollination in either the production of extra pods or seeds/pod. All of the extra pods set after hand pollination of the apetalous line were carried through to maturity, indicating that pod abortion post-pollination did not contribute to the high number of aborted pods produced by the apetalous A lines.

Some apetalous lines also appeared to have low female fertility, producing a high number of aborted ovules even after hand pollination, and the abortion of seed before the final harvest. It was suggested that this may be associated with the presence of radish genetic material introduced in the *Ogura* CMS system as reported by Delourme and Eber (1992). Lines 1806 and 2809, which had the lowest levels of female fertility, shared a common genetic background in being derived from NSW lines, strongly suggesting a genetic basis to the problem. The performance of the other apetalous lines indicated that low female fertility was not linked to the apetalous character, and that better pollination would lead to higher yields from those apetalous lines.

The absence of petals and restricted stamen development of the apetalous A line flowers were found to be responsible for some flowers failing to open successfully. Flowers with styles caught in the sepals were highly unlikely to be pollinated while the stigma was receptive. The occurrence of caught styles was reported in the original source of the *Ogura* CMS, *Raphanus* and other *Ogura* CMS lines (Polowick and Sawhney, 1987). However the effect which this may have on final yield has not previously been discussed.

Flowers with caught styles appeared to be one of the reasons for the high number of aborted pods observed on apetalous A line plants. There were differences between the apetalous A lines in the proportion of flowers with caught styles, which appeared to explain why some lines had more aborted pods than others in field trials. The occurrence of caught styles was further influenced by the temperature under which the plants were grown. The lines in which flowers opened successfully under a given temperature treatment had stamens with relatively well developed filaments.

These filaments were quite long relative to the style, even though the anthers they supported did not develop, and the style in turn was quite robust.

Apetalous B line flowers did not show any evidence of suffering from caught styles as the presence of fully developed stamens assisted in forcing the sepals open and releasing the style. Similarly the presence of petals in the 20894 A line flowers resulted in flowers always opening successfully.

Given these findings it should be possible to select apetalous A lines having flowers with well developed filaments and robust styles that are able to successfully open a high percentage of flowers.

When considering the number of factors which have been shown to influence the successful pollination of an A line apetalous flower, it was not surprising that the yields of the apetalous A lines varied considerably from season to season, while those of the petalled A line remained relatively constant. The apetalous lines appeared to be especially susceptible to periods of high temperature during flowering, which may have been due to several reasons. The effect that the apetalous character had on the temperature experienced by the crop canopy has not been previously considered, and was not investigated in this study. However it is likely that removing the reflective effect of petals would increase the temperature experienced by the apetalous flowers. Morrison (1993) demonstrated that heat stress affected both the male and female reproductive organs, although female fertility was affected to a greater extent. It was also reported that there were significant differences in the susceptibility to high temperature between the two cultivars used in the trials, indicating that there may be genetic variability for heat stress sensitivity. Such variability may explain why the apetalous lines 1806 and 2809 retained a low percentage of seeds/pod, even after hand pollination.

The requirement of apetalous flowers for a greater number of bee visits for pod and seed set also makes them more susceptible to conditions which have an adverse effect on pollination vectors, in this study mainly honey bees. Such conditions include cold, windy weather which restricts bee activity, or hot weather which focuses bee behaviour on reducing the hive temperature rather than collecting nectar and pollen.

The logarithmic regression coefficients for adjusted seed yield as a function of row number from the pollen source, varied quite widely between different apetalous A lines and seasons. In contrast, the petalled line had less variation between seasons, behaving in a similar manner to the petalled *Polima* A lines investigated by Pinnisch and McVetty (1990). They reported that total seed yield in relation to distance from the pollen source was fairly constant over a variety of environmental factors, including location and year.

Variation between seasons for the apetalous lines was even more apparent when the logarithmic regression of the number of seeds/m^2 with distance from the pollen source was investigated (figure 6.6, page 138), and highlighted the greater environmental influence on seed set experienced by the apetalous A lines. These trials did not show conclusively what pollination conditions were optimal, but the results from the 94/95 season clearly indicated that high temperatures during flowering should be avoided where possible. While the avoidance of high temperature is also necessary in *Polima* A lines to prevent male sterility breakdown, in the *Ogura* lines 100% hybrid seed will still be assured, though yields will most probably be reduced.

From the average logarithmic regression relationships for all the apetalous lines from the 95/96 and 96/97 seasons, economically acceptable yields were obtained up

to 16 rows from the pollen parent. This was based on the equivalent of 500 kg/ha of hybrid seed being set as the minimum acceptable yield as per Pinnisch and McVetty (1990), and is in accord with the experience of hybrid seed production in Tasmania.

This result compared favorably with the recommendations made by Pinnisch and McVetty (1990), who determined that 6 rows of A line would be the maximum distance from the pollen source in *Polima* hybrid seed production, before levels of hybrid seed fell to unacceptable levels. This recommendation was based on the percentage of hybrid seed produced, not total seed yield. Seed hybridity was also found to decline significantly with increasing row number as a result of the breakdown of male sterility, which occurred when the *Polima* A lines produced blisters of pollen.

With *Ogura* lines having more reliable male sterility that those with *Polima* CMS, the problem should be more of seed yield than levels of hybridity.

As a result of the layout and design of trials conducted during the course of the present research, there is little doubt that some cross-pollination would have occurred between different lines. This was unavoidable if these trials were to be kept within manageable size limits. However, the regression analysis conducted indicated that this did not have a major effect on total seed yield, as the relationship with increasing row number was significant for all the apetalous lines despite pollen from other lines being available in a non-directional manner.

The late flowering line, 1806, flowered in isolation in the 96/97 season and the logarithmic regression for adjusted seed yield and row number produced the highest r^2 value obtained of 0.72. Line 1806 produced just under 500 kg/ha of seed up to 15

rows from the pollen source, indicating that the 16 row maximum distance suggested previously would be a reasonable recommendation.

While Pinnisch and McVetty (1990) used linear regression to determine the maximum number of A line rows, logarithmic regression was found to fit the data more accurately in these trials. If the average linear regression coefficients for the apetalous lines from the 95/96 and 96/97 seasons were used to determine the maximum number of A line rows, economic yields were produced up to 14 rows from the pollen source.

Observations made of the interactions of honey bees with apetalous male sterile flowers suggest that it would be of great benefit to use higher bee populations than were used in these trials to maximise the number of bee visits a flower receives while still viable. It is also vitally important to prolong the flowering of the pollen source by cutting back a portion of the male fertile plants during early flowering, or by using a staggered planting system so that some males are flowering over an extended period. This would enable more seed to be set on secondary and tertiary branches of A line plants, which in the case of the apetalous A lines continued flowering after the respective B lines had finished.

In all the trials and experiments conducted in this study there was no evidence of the *Ogura CMS* system failing and male sterile plants producing pollen. This has also been the experience with other *Ogura* lines used by Pacific Seeds (A. Easton, Pacific Seeds Toowoomba, Qld., pers. comm.). Therefore with the implementation of proper isolation distances 100% hybrid seed should be assured with this system.

The feasibility of apetalous hybrids will ultimately depend on the performance of the F_1 generation, which is yet to be determined. However based on the results obtained

Conclusions

• Apetalous hybrid seed production is possible, but yields are likely to be lower and more variable than with conventional petalled seed.

• Lower seed yield in apetalous A lines as compared to the corresponding apetalous B lines or petalled A line was due to poorer pod and seed set.

• Lack of pollination was the main contributing factor. This was a function of poor flower opening in some cases, where the stigma and style were caught in the sepals during flower opening of the apetalous plants which were also male sterile, that is without anthers or petals to push the flower open. Incidence of caught styles varied with temperature and genotype.

• Bee behaviour contributed to the poor pollination, by "sideworking" apetalous flowers and hence avoiding deposition of pollen on the stigma surface, even though the flowers appeared equally as attractive to bees as conventional flowers.

• More bee visits were necessary for effective pollination on apetalous male sterile flowers, and hence seed set varied in a logarithmic pattern with distance or number of A line rows from the B line, the pollen source. Increased bee numbers and fewer rows of A lines between B lines are needed, although economic seed yields of 500 kg/ha should be able to be produced with up to 16 A line rows.

• Female fertility (proportion of ovules which responded to pollination by producing seed) varied between lines but did not appear to be a function of the apetalous character.

• Seed and pod set, and hence the economics of seed production, are likely to be improved by selection for larger filaments and more robust styles to promote flower opening, by forcing open sepals in the absence of petals and functional anthers.

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Appendix A

Chapter 2 Statistical Data.

Table 2.3

Analysis of variance for plant numbers.

Plants/m^2 N: 81 MULTIPLE R: 0.940 SQUARED MULTIPLE R: 0.884
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TREATMENT	212760.244	13	16366.173	37.730	0.000
REPLICATE	359.101	2	179.550	0.414	0.663
ERROR	28195.461	65	433.776		

3 CASES DELETED DUE TO MISSING DATA.

Two from 30205(HIGH) treatment, and one from 1806(HIGH).

Table 2.4

Analysis of variance for yield and yield components.

Yield kg/ha N: 42 MULTIPLE R: 0.848 SQUARED MULTIPLE R: 0.720
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REPLICATE	3412150.375	2	1706075.187	7.167	0.000
TREATMENT	3217418.820	13	247493.755	2.490	0.023
ERROR	2583941.080	26	9382.349		

Productive pods/m^2 N: 39 MULTIPLE R: 0.926 SQUARED MULTIPLE R: 0.858
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REPLICATE	9113699.003	2	4556849.502	8.466	0.002
TREATMENT	.600785E+08	13	4621419.984	8.586	0.000
ERROR	.123803E+08	23	538272.947		

3 CASES DELETED DUE TO MISSING DATA.
1806(HIGH), 2807 and 30205(HIGH)

Aborted pods/m^2 N: 40 MULTIPLE R: 0.949 SQUARED MULTIPLE R: 0.900
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REPLICATE	34066.331	2	17033.165	0.133	0.876
TREATMENT	.274526E+08	13	2111735.207	16.471	0.000
ERROR	3077113.035	24	128213.043		

2 CASES DELETED DUE TO MISSING DATA.
One from 1803 and one from 2807.

Seeds/pod N: 40 MULTIPLE R: 0.798 SQUARED MULTIPLE R: 0.636
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REPLICATE	71.897	2	35.949	1.848	0.179
TREATMENT	747.744	13	57.519	2.957	0.010
ERROR	466.791	24	19.450		

2 CASES DELETED DUE TO MISSING DATA.
30250 and 30205(HIGH)

Individual Seed Weight. N: 126 MULTIPLE R: 0.869 SQUARED MULTIPLE R: 0.755
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REPLICATE	0.565	2	0.282	7.643	0.001
TREATMENT	11.971	13	0.921	24.930	0.000
ERROR	4.063	110	0.037		

Table 2.5

Analysis of variance for yield of spring sown trial.

Yield kg/ha N: 38 MULTIPLE R: 0.763 SQUARED MULTIPLE R: 0.582
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
REPLICATE	47432.490	2	23716.245	0.440	0.649
TREATMENT	1532842.839	13	117910.988	2.189	0.051
ERROR	1185208.341	22	53873.106		

4 CASES DELETED DUE TO MISSING DATA.
30205, 1806, 1806(LOW), 2809.

Table 2.6

Analysis of variance for yield and individual seed weight of spring sown hybrid trial.

Yield kg/ha N: 28 MULTIPLE R: 0.933 SQUARED MULTIPLE R: 0.870

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	6061421.583	6	1010236.931	21.452	0.000
REPLICATE	244734.569	1	244734.569	5.197	0.034
ERROR	941871.931	20	47093.597		

Individual seed weight N: 28 MULTIPLE R: 0.982 SQUARED MULTIPLE R: 0.965
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	15.543	6	2.590	92.002	0.000
REPLICATE	0.015	1	0.015	0.536	0.473
ERROR	0.563	20	0.028		

Figure 2.5.

Data and S.E of pods/plant used to construct figure 2.5.

	15/12/94	*29/12/94*	*13/1/95*
1803	10.2	178.7	272.3
1806	4.3	217.3	117.8
1811	67	411.8	70.0
2804	7.2	118.4	202.5
2807	4.3	177.8	102.7
2809	1.2	127.0	231.5
20894	11.8	107.8	124.3
S.E.	*4.2*	*51.01*	*30.3*
n	*6*	*6*	*6*

Appendix B

Chapter 3 Statistical Data.

Analysis of variance for yield and components used to construct table 3.3.

TRIAL 1
Yield N: 46 MULTIPLE R: 0.831 SQUARED MULTIPLE R: 0.690
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	.370808E+08	2	.185404E+08	47.948	0.000
ERROR	.166269E+08	43	386673.170		

Due to incomplete results analysis was based on individual row yields.

Individual Seed weight N: 46 MULTIPLE R: 0.730 SQUARED MULTIPLE R: 0.533
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	3.500	2	1.750	24.504	0.000
ERROR	3.071	43	0.071		

TRIAL 2

YIELD N: 16 MULTIPLE R: 0.964 SQUARED MULTIPLE R: 0.930
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	.137260E+08	3	4575317.634	48.072	0.000
REPLICATE	149914.160	1	149914.160	1.575	0.235
ERROR	1046929.053	11	95175.368		

Data for Yield Analysis. Trial 2

Line	Replicate	Yield kg/ha
1803	1	678.61
1803	1	756.14
1803	2	947.4
1803	2	967.23
1806	1	962.2
1806	1	770.62
1806	2	903.04
1806	2	855.01
2807	1	866.86
2807	1	979.67
2807	2	742.36
2807	2	865.28
20894	1	2519.26
20894	1	2832.79
20894	2	3877.58
20894	2	2757

Individual seed weight N: 16 MULTIPLE R: 0.971 SQUARED MULTIPLE R: 0.943
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	10.888	3	3.629	59.965	0.000
REPLICATE	0.084	1	0.084	1.390	0.263
ERROR	0.666	11	0.061		

Productive pods/m^2 N: 133 MULTIPLE R: 0.438 SQUARED MULTIPLE R: 0.192
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	.233975E+08	3	7799182.722	1.056	0.370
REP	.133357E+08	1	.133357E+08	1.805	0.181
ERROR	.945734E+09	128	7388545.243		

* some samples were lost due to mice damage during storage.

Aborted pods/m^2 N: 179 MULTIPLE R: 0.702 SQUARED MULTIPLE R: 0.493
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	.653693E+10	3	.217898E+10	54.852	0.000
REPLICATE	.398118E+08	1	.398118E+08	1.002	0.318
ERROR	.691215E+10	174	397250E+08		

Number of seeds/pod N: 54 MULTIPLE R: 0.970 SQUARED MULTIPLE R: 0.941
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	3040.931	2	1520.465	355.943	0.000
BRANCHES	15.592	2	7.796	1.825	0.173
LINE*BRANCHES	22.033	4	5.508	1.290	0.288
ERROR	92.224	45	4.272		

Analysis of variance for components used to construct table 3.4.

Branches/Plant N: 54 MULTIPLE R: 0.837 SQUARED MULTIPLE R: 0.701
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	2945.212	2	1472.606	41.498	0.000
BRANCH	138.913	2	7.79669.456	1.957	0.153
LINE*BRANCH	664.285	4	166.071	4.680	0.003
ERROR	1596.864	45	35.486		

Productive pods on mainstem and primary branches
N: 90 MULTIPLE R: 0.695 SQUARED MULTIPLE R: 0.483
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	19568.467	2	9784.233	22.221	0.000
ROW	674.800	5	334.960	0.761	0.581
LINE*ROW	8403.533	10	840.353	1.909	0.058
ERROR	31702.800	72	440.317		

Productive pods on secondary branches
N: 90 MULTIPLE R: 0.641 SQUARED MULTIPLE R: 0.411
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	17300.689	2	8650.344	10.156	0.000
ROW	2920.356	5	584.071	0.686	0.636
LINE*ROW	22592.511	10	2259.251	2.652	0.008
ERROR	61325.600	72	851.744		

Productive pods on tertiary branches
N: 90 MULTIPLE R: 0.480 SQUARED MULTIPLE R: 0.230
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	12192.289	2	6096.144	2.958	0.058
ROW	10250.722	5	2050.144	0.995	0.427
LINE*ROW	21913.311	10	2191.331	1.063	0.402
ERROR	148384.000	72	2060.889		

Aborted pods on mainstem and primary branches.
N: 90 MULTIPLE R: 0.759 SQUARED MULTIPLE R: 0.576
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	74055.267	2	37027.633	41.811	0.000
ROW	7181.700	5	1436.340	1.622	0.165
LINE*ROW	5443.133	10	544.313	0.615	0.797
ERROR	63762.800	72	885.594		

Aborted pods on secondary branches
N: 90 MULTIPLE R: 0.672 SQUARED MULTIPLE R: 0.451
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	172504.800	2	86252.400	21.886	0.000
ROW	23680.633	5	4736.127	.202	0.317
LINE*ROW	37244.667	10	3724.467	0.945	0.498
ERROR	283748.000	72	3940.944		

Aborted pods on tertiary branches
N: 90 MULTIPLE R: 0.654 SQUARED MULTIPLE R: 0.427
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	858091.756	2	429045.878	17.422	0.000
ROW	142070.322	5	28414.064	1.154	0.340
LINE*ROW	322177.844	10	32217.784	1.308	0.243
ERROR	1773166.400	72	24627.311		

Individual Seed weight. N: 54 MULTIPLE R: 0.880 SQUARED MULTIPLE R: 0.775
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	44.321	2	22.160	7.164	0.000
BRANCHES	5.751	2	2.876	8.716	0.001
LINE*BRANCHES	1.095	4	0.274	0.830	0.513
ERROR	14.848	45	0.330		

Appendix C

Chapter 4 Statistical Data.

Analysis of variance for yield and components used to construct table 4.2.

YIELD N: 20 MULTIPLE R: 0.990 SQUARED MULTIPLE R: 0.981
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	.201549E+08	4	5038736.790	177.180	0.000
REP	350995.162	1	350995.162	12.342	0.003
ERROR	398139.439	14	28438.531		

Individual seed weight. N: 20 MULTIPLE R: 0.950 SQUARED MULTIPLE R: 0.902
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	7.367	4	1.842	31.612	0.000
REP	0.131	1	0.131	2.252	0.156
ERROR	0.816	14	0.058		

Productive pods/plant N: 50 MULTIPLE R: 0.488 SQUARED MULTIPLE R: 0.238
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	113961.720	4	28490.430	2.395	0.066
TREAtMENT	8013.780	1	8013.780	0.674	0.417
LINE*TREATMENT	26495.720	4	6623.930	0.557	0.695
ERROR	475800.800	40	11895.020		

Aborted pods/plant N: 50 MULTIPLE R: 0.696 SQUARED MULTIPLE R: 0.484
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	1629532.280	4	407383.070	8.100	0.000
TREATMENT	8013.780	1	8013.780	0.159	0.692
LINE*TREATMENT	250946.120		62736.530	1.247	0.307
ERROR	2011876.400	40	50296.910		

Seeds/pod N: 50 MULTIPLE R: 0.917 SQUARED MULTIPLE R: 0.841
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	1703.521	4	425.880	51.280	0.000
TREATMENT	7.984	1	7.984	0.961	0.333
LINE*TREATMENT	42.643	4	10.661	1.284	0.293
ERROR	332.199	40	8.305		

Analysis of variance for components used to construct table 4.3.

In order for analysis of variance to be conducted the percentage data underwent an arcsine transformation as this type of data forms a binomial rather than a normal distribution. This transformation results in the data having a distribution which is nearly normal. (For more information refer to Zar (1996))

The transformed data used to calculate least significant differences of the percentage of productive pods set from potential pod sites, presented in table 4.5, chapter 4.

Percentage of Productive Pods Set.

	Sample A		Sample B		Sample A+B	
	Pollinated	*Control*	*Pollinated*	*Control*	*Pollinated*	*Control*
1803	52.83	44.01	60.86	52.77	56.85	48.39
1806	64.83	54.78	62.03	48.71	63.43	51.75
2807	60.91	43.81	59.30	43.22	60.11	43.51
2809	55.21	39.09	50.04	43.11	52.62	41.10
20894	59.21	62.64	73.06	64.47	66.13	63.56
		lsd=10.90			lsd=7.71	
Means	*58.60*	*48.87*	*61.06*	*50.46*	*59.83*	*49.66*
		lsd=4.48			lsd=3.45	
Combined Means	*53.73*		*55.76*			

Percentage of Productive Pods
N: 100 MULTIPLE R: 0.754 SQUARED MULTIPLE R: 0.568
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	3707.218	4	926.804	2.350	0.000
TREAT	2584.530	1	2584.530	34.440	0.000
SAMPLE	102.524	1	102.524	1.366	0.246
LINE*TREAT	529.863	4	132.466	1.765	0.144
LINE*SAMPLE	663.817	4	165.954	2.211	0.075
TREAT *SAMPLE	4.781	1	4.781	0.064	0.801
LINE*TREAT *SAMPLE	296.820	4	74.205	0.989	0.419
ERROR	6003.572	80	75.045		

Analysis of variance for components used to construct table 4.4.

Total ovules/pod. Sample A

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	449.109	4	112.277	17.695	0.000
TREAT	32.000	1	32.000	5.043	0.030
LINE*TREAT	46.136	4	11.534	1.818	0.144
ERROR	253.808	40	6.345		

Analysis of variance for components used to construct table 4.5. .

Fertile ovules and seeds/pod
N: 100 MULTIPLE R: 0.822 SQUARED MULTIPLE R: 0.675

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	1872.467	4	468.117	21.907	0.000
TREAT	805.027	1	805.027	37.674	0.000
SAMPLE	91.757	1	91.757	4.294	0.041
LINE*TREAT	442.033	4	110.508	5.172	0.001
LINE*SAMPLE	245.436	4	61.359	2.871	0.028
TREAT*SAMPLE	36.325	1	36.325	1.700	0.196
LINE*TREAT*SAMPLE	59.575	4	14.894	0.697	0.596
ERROR	1709.475	80	21.368		

The arcsine transformed data used to calculate least significant differences in female fertility presented in chapter 4, table 4.6.

	Percentage Female Fertility	
	Sample A	Sample B
1803	56.6	47.5
1806	50.3	38.5
2807	52.4	26.8
2809	45.88	29.7
20894	58.4	57.3
	lsd (0.05)=13.1	
Means	*52.7*	*40.00*
	lsd(0.05)=4.14	

Female fertility Sample A and B.
N: 100 MULTIPLE R: 0.797 SQUARED MULTIPLE R: 0.635

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	7953.645	4	1988.441	18.334	0.000
TREAT	3106.358	1	3106.358	28.642	0.000
SAMPLE	290.410	1	290.410	2.678	0.106
LINE*TREAT	2329.823	4	582.456	5.371	0.001
LINE*SAMPLE	1041.032	4	260.258	2.400	0.057
TREAT*SAMPLE	65.304	1	65.304	0.602	0.440
LINE*TREAT*SAMPLE	334.229	4	83.557	0.770	0.548
ERROR	8676.248	80	108.453		

Analysis of variance for components used to construct table 4.7.

Bee Numbers on different lines, over three observation periods.
N: 15 MULTIPLE R: 0.927 SQUARED MULTIPLE R: 0.858
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	1.536	4	0.384	0.976	0.471
OBSERVATION	17.542	2	8.771	22.302	0.001
ERROR	3.146	8	0.393		

Proportion of successful bee visits on different lines, arcsine transformed data.
N: 15 MULTIPLE R: 0.927 SQUARED MULTIPLE R: 0.858
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	2190.425	4	547.606	23.064	0.002
ERROR	118.713	5	23.743		

Arcsine transformed data of successful bee visits

	Transformed Mean
1803	36.2
1806	44.6
2807	34.6
2807	22.7
20894	67.2
S.E.	3.4
n	2

Appendix D

Chapter 5 Statistical Data.

Data of floral organ size used to construct figure 5.2.

			Stamens		
Temperature	*Line*	*Sepal length*	*Long*	*Short*	*Gynoecium*
HH	*1803A*	5.61	3.46	3.15	6.07
	1803B	6.91	8.73	7.44	8.99
SH	*1803A*	6.82	7.39	6.43	8.23
	1803B	8.59	9.23	8.55	8.83
HH	*1806A*	6.72	4.68	3.67	8.61
	1806B	7.49	9.68	7.97	9.3
SH	*1806A*	7.1	5.28	4.34	8.9
	1806B	8.68	9.81	8.11	9.13
HH	*2807A*	5.76	4.25	3.56	6.64
	2807B	6.58	8.47	7.81	8.52
SH	*2807A*	6.87	4.82	4.12	9.03
	2807B	8.8	9.48	8.72	9
HH	*2809A*	5.82	3.46	3.1	6.4
	2809B	6.91	8.66	7.06	8.83
SH	*2809A*	7.43	6.05	5.13	8.7
	2809B	8.23	10.48	8.88	8.75
HH	*20894A*	6.13	5.73	4.34	8.67
	20894B	7.11	9.59	7.6	9.07
SH	*20894A*	7.76	7.14	5.68	8.04
	20894B	10.09	10.45	7.88	9.49
	S.E.	*0.32*	*0.59*	*0.49*	*0.81*
	lsd(0.05)	*0.32*	*0.59*	*0.49*	*0.81*

SEPAL LENGTH N: 300 MULTIPLE R: 0.933 SQUARED MULTIPLE R: 0.871
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TREAT	175.920	1	175.920	80.442	0.000
LINE	168.442	9	18.716	93.669	0.000
TREAT*LIN E	32.756	9	3.640	18.215	0.000
ERROR	55.946	280	0.200		

LONG STAMEN LENGTH N: 300 MULTIPLE R: 0.947 SQUARED MULTIPLE R: 0.897
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TREAT	135.113	1	135.113	198.791	0.000
LINE	1436.635	9	159.626	234.857	0.000
TREAT*LIN E	91.375	9	0.153	14.938	0.000
ERROR	190.308	280	0.680		

SHORT STAMEN LENGTH N: 286 MULTIPLE R: 0.960 SQUARED MULTIPLE R: 0.921
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TREAT	106.183	1	106.183	283.486	0.000
LINE	1001.899	9	111.322	297.206	0.000
TREAT*LIN E	53.010	9	5.890	15.725	0.000
ERROR	99.633	266	0.375		

Gynoecium Length N: 298 MULTIPLE R: 0.664 SQUARED MULTIPLE R: 0.440
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TREAT	36.456	1	36.456	30.499	0.000
LINE	139.746	9	15.527	12.990	0.000
TREAT*LIN E	87.158	9	9.684	8.102	0.000
ERROR	332.294	278	1.195		

Data of pollen loads used to construct table 5.2.

Number of Pollen Grains N: 100 MULTIPLE R: 0.636 SQUARED MULTIPLE R: 0.404
ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
LINE	32421.300	4	8105.325	16.123	0.000
ERROR	47758.700	95	502.723		

Appendix E

Chapter 6 Statistical Data.

Logarithmic regression equations and R^2 values for unadusted yield used to construct table 6.1 and figures 6.2 and 6.3.

		Logarithmic regression equations	R^2
1995/96	1803	y = 1625.2 - 1106.2 log(x)	0.43
	1806	y = 1199.7 - 471.0 log(x)	0.25
	2807	y= 1774.9 - 1313.3 log(x)	0.52
	20894	y= 4530.3 - 1877.89 log(x)	0.22
1996/97	1803	y = 2603.0 - 1026.6 log(x)	0.24
	1806	y = 3171.3 - 2198.0 log(x)	0.65
	2807	y = 1038.8 - 623.7 log(x)	0.18
	2809	y = 2682.0 - 1726.0 log(x)	0.53
	20894	y = 4572.2 - 1152.9 log(x)	0.08

Linear regression equations and R^2 values for adusted yield used to construct table 6.2 and figures 6.4 and 6.5.

		Linear regression equations	R^2
1995/96	1803	y = 1361.6 - 90.45x	0.28
	1806	y = 1155.3 - 56.4x	0.39
	2807	y = 1473.0 - 108.3x	0.35
	20894	y = 4043.1 - 174.2x	0.21
1996/97	1803	y = 2271.9 -74.73x	0.28
	1806	y = 2513.3 - 149.2x	0.54
	2807	y = 851.2 - 43.7x	0.16
	2809	y = 2096.8 - 108.8x	0.37
	20894	y = 3990.1 - 75.4x	0.09

Logarithimic regression equations and R^2 values for adusted yield used to construct table 6.2 and figures 6.4 and 6.5.

		Logarithmic regression equations	R^2
1995/96	1803	y = 1618.9 - 1155.7 log(x)	0.44
	1806	y = 1186.1 - 532.6 log(x)	0.31
	2807	y = 1767.9 - 1367.9 log(x)	0.53
	20894	y = 4482.6 - 2130.1 log(x)	0.29
1996/97	1803	y = 2593.0 - 1137.7 log(x)	0.37
	1806	y = 3162.7 - 2281.3 log(x)	0.72
	2807	y = 1037.3 - 664.6 log(x)	0.21
	2809	y = 2672.6 - 1790.0 log(x)	0.58
	20894	y = 4456.9 - 1449.2 log(x)	0.19

Linear regression equations and R^2 values for seed numbers per row used to construct table 6.2 and figures 6.4 and 6.5.

		Linear regression equations	R^2
1995/96	1803	y = 209.0 - 15.2x	0.34
	1806	y = 219.0 - 12.6x	0.53
	2807	y = 311.1 - 25.8x	0.38
	20894	y = 971.7 - 45.0x	0.25
1996/97	1803	y = 374.8 - 15.2x	0.39
	1806	y = 380.1 - 23.3x	0.55
	2807	y = 137.1 - 7.2x	0.16
	2809	y = 293.0 - 15.7x	0.38
	20894	y = 875.1 - 25.6x	0.19

Logarithimic regression equations and R^2 values for seed numbers per row used to construct table 6.2 and figures 6.4 and 6.5.

		Logarithmic regression equations	R^2
1995/96	1803	y = 252.6 - 193.0 log(x)	0.50
	1806	y = 231.3 - 126.3log(x)	0.48
	2807	y = 374.5 - 315.8log(x)	0.56
	20894	y = 1078.5 - 542.6 log(x)	0.34
1996/97	1803	y = 442.6 - 234.7 log(x)	0.53
	1806	y = 481.2 - 356.4 log(x)	0.74
	2807	y = 167.0 - 108.6 log(x)	0.21
	2809	y = 376.7 - 255.6 log(x)	0.58
	20894	y = 998.7 - 406.2 log(x)	0.31